

British Forests Need Lord Rothschild

THE British Government has done its best, in the past few months, to cut the figure of a resolute and hard-headed manager of research and development, eager always to balance the cost of research against the potential benefits. This, at least, is the motive for its new policy on the research councils, derived for all practical purposes from Lord Rothschild's recipe for reorganization. Given the zeal with which the campaign against the research councils has been prosecuted, however, it is unseemly that so little has been done to apply the Rothschild principles consistently. Defence research and development remain scandalously immune from tests like these. So, too, do much smaller programmes of publicly supported research, that carried out by the Forestry Commission, for example. Yet the latest annual report of the Forestry Commission (HMSO, £0.75) shows not merely that the total cost of the Forestry Commission's research increased between 1971 and 1972 by 25 per cent to a total of £1.28 million but also that this patient work, sometimes valuable, often hum-drum and occasionally pointless, is undertaken on behalf of an industry whose commercial value is zero or even less.

The future of the Forestry Commission's policy on research cannot be divorced from the future of its commercial operations. In June last year, the government fired a warning shot across the commission's bows by publishing a consultative document which raised, not before time, the question of the scale on which the Forestry Commission should in future operate (*Forestry Policy*, June 1972, HMSO, £0.18). Originally, in 1919, the commission was asked to make a contribution to the strategic independence of the United Kingdom by buying land and planting trees thereon. But it has been plain, since 1958, that this is by no means a compelling justification for the continued existence of the commission—British trees at present account for only six per cent of total consumption and there are, in any case, other strategic uses that might be made of the 0.75 million hectares which the commission at present owns. This is why the government has increasingly sought to justify the commission's continued existence by saying that it is a kind of social service, providing jobs in parts of Britain where unemployment is high and the threat of depopulation is severe and, more recently, both the government and the commission have been arguing that the national forests are potentially valuable as means of recreation.

Whatever the justification for the Forestry Commission, there is no doubt that its books are in a mess. The commission functions by borrowing money from the government to finance the purchase of land and the cost of planting this with trees and has dutifully, for the past half century, kept a record of the cumulative cost of its forest building, with interest calculated at a rate which averages five per cent or thereabouts. By March 31, 1972, the total cost of forests still growing trees amounted to £405 million—a figure now recognized to be far in excess of the commercial value of the timber that will eventually be

harvested. The 182,000 hectares of forest in which production had begun last March are, for example, estimated to have cost £130 million, but the commission's own estimate of the value of the timber is merely £88 million. Since the commission has used a discount rate of only five per cent in reaching this conclusion, it must be acknowledged that even this estimate of the present value of the forests is wildly optimistic—on the open market, they would not fetch a fraction of £88 million. Another way of putting this, also apparent from the commission's accounts, is to say that in the year to March 1972, it cost £5.4 million in direct expenses to harvest £7.5 million worth of wood from forests whose cumulative value was £4.5 million. In other words, as the commission has become, almost by accident, a great landowner it can make a decent return on its operations only by ignoring the cost of having grown its trees.

The consultative document *Forestry Policy*, to its credit, acknowledges that this state of affairs cannot be allowed indefinitely to persist. So far so good. But it is hard to see why the document blandly proposes that what it calls the "notional liabilities" of the Forestry Commission should now be written down to such a level that the commission will in future be able to earn three per cent a year on what will then be its investment, compared with the target of ten per cent a year fixed for most other public enterprises. For whatever the government may say about the notional character of the Forestry Commission's accounts, the truth is that the economic resources which have been forgone in the past half century are at least equal to the £404 million which represents the value of growing forests in the commission's books, may actually be more fairly represented by the £474 million given as the commission's present liabilities and would in any case be much greater if a realistic rate of interest had been charged, notionally or otherwise. If a large proportion of this capital is now to be written off, it should be openly acknowledged that the sum involved is a measure of past errors of judgment and in particular of the Forestry Commission's pigheadedly bullish view of the scale of operations which would be justified.

The government goes only some of the way to recognizing what the implications for the future should be. On the basis of a cost-benefit study carried out by government economists (*Forestry in Great Britain*, HMSO, £1.25), *Forestry Policy* says that "new planting cannot be justified by the direct and quantifiable benefits alone" and goes on to say that continued justification of the Forestry Commission's existence must depend on such things as the contribution which jobs in forestry make to preventing the depopulation of some rural parts in Britain and the value of its forests as places for recreation. The truth is, however, that providing jobs in forestry is an expensive business—the direct cost to the Exchequer ranges from £16,500 per job in Wales to £23,000 per job in the south of Scotland and is calculated to be an average of £10,000 per job in real terms (which implies making allowance for



the likelihood that many of those now employed in forestry would be drawing unemployment benefit if the Forestry Commission were to vanish overnight).

In all the circumstances, it is hard to see why the government leaps blandly to the conclusion that the best policy for the years ahead is to restrict new planting of state forests to 22,000 hectares a year, with the proviso that the policy should be reviewed every three years in the light of changing employment patterns. Recognizing that the Forestry Commission's labour force of 9,055 is already dwindling, that hill farming may become substantially more profitable once the agricultural policy of the European Community comes into effect but that there are many small rural communities which have been reduced by the policies of the past half century to become dependent on forestry, a much more radical approach is called for. First, the Forestry Commission should be prevented from acquiring new land except when it can be demonstrated that a new forest will have a direct benefit on local unemployment at a cost which is supportable. It would be entirely proper to ask that the total labour force should continue to contract at five per cent a year. Second, the Forestry Commission should be instructed to sell off the forests it has grown whenever there is a chance of recovering the public money now locked up in nationalized trees. Third, the commission should be compelled to adopt the policy, advocated in the consultative document, of replanting only the most productive parts of cleared forests, letting the rest seed themselves naturally. Fourth, some means should be found of exploiting more imaginatively than the Forestry Commission can the opportunities for recreation which, after decades of devotion to impenetrable barbed wire fences, the commission now appears to recognize as its best hope of survival.

On recreation, both the commission and the government are naive, to say the best of it. The present value of the recreational value of the state forests is put at £1.5 million a year, chiefly on the basis of the amount of time which visitors at present spend in state forests and on a notional value of the amenity bestowed on them by the sight of trees. There is enough muttering in the cost/benefit analysis and the Forestry Commission's report to suggest that everybody concerned is anxious to increase the use of forests and that the Forestry Commission itself will soon start charging larger sums to those who pitch their tents overnight at authorized camping sites. An especially impenetrable report on a research project commissioned by the Forestry Commission at the University of Reading (see *Forest Research 1972*, HMSO, £1.60, also published last week) talks of "the recreational supply capability" of forests and of the "unrealized recreational potential" of the commission's holdings and promises the construction of a "recreation capability index" for half a dozen randomly selected forests. Nobody, however, seems seriously to have considered how much recreational capability potential has been destroyed, in recent decades, by the commission's policy of putting forests where people used to walk or even the commercial question of whether it might not be preferable to forget about the trees in many national forests and to turn over the land to organizations, public or private, which would manage them as recreational parks.

Against this background, the scale of the Forestry Commission's research programme is a great affront.

Much of it is concerned with the collection and testing of seed and the improvement of germination, but who would suggest that even outstanding success will make the forests profitable? There is something to be said for reducing labour costs by the new techniques for planting trees developed by the research division, paradoxical though this may be if the chief justification for the forests is that they provide employment. The most interesting part of the Forestry Commission's research programme is the genetics, the physiology and pathology of forest trees, but there is a case for thinking that this could be done at least as well within the research councils and with the help of the universities, to which the Forestry Commission puts out less than two per cent of its expenditure on research. Unhappily, however, *Forestry Policy* has nothing at all to say about research. The chances are that if even Lord Rothschild were asked to say what he thinks should be done, he would find himself proposing either that the programme should be discontinued for lack of commercial justification or that the more interesting parts of it should be transferred from the Forestry Commission to the research councils on the grounds that it has very little relevance to the immediate future.

White House Muddle

THE outlook for American science and technology is gloomy. The departure of Dr Edward David from the Office of Science and Technology (see page 82) is one ominous sign of the inadequacy of the science budget for the coming financial year. President Nixon's determination to restrict the level of federal expenditure is another. The truth about the budget should be known in a few weeks, when the Administration's proposals for the financial year beginning in July will be published, although the confusion which stems from the way in which Congress's version of the Health, Education and Welfare Bill is regularly vetoed by the President will probably ensure that agencies such as the National Science Foundation will have no idea what they have to spend until the financial year is half gone. (The budget for the current year has not yet been settled.) In the long run, however, it is more serious that the Administration seems to have drifted into a muddle about its arrangements for the administration of science and technology in the United States. And, as always, it appears to have no clear idea of its objectives.

The first thing to be said, in the wake of Dr David's departure, is that it is less important, for the scientific community in the United States, that the Office of Science and Technology should be preserved in its traditional form than that the Administration should make a clear commitment to basic research and in particular to the National Science Foundation, the National Institutes of Health and to the National Aeronautics and Space Administration and the Atomic Energy Commission, which remain substantial sponsors of long-term research. For one thing, the federal government is the only means by which American universities can train graduate students and contribute to the scholarship on which the technology of the 1980s must be based. Ever since 1967, when President Johnson lightly brushed aside a demand from the National Science Board that graduate education and

scholarship in science and technology should be recognized as a federal responsibility, the Administration has been shillyshallying. Agencies such as the National Science Foundation have not helped by their trendy eagerness to be more relevant, whatever that may mean. What is needed now is some mechanism by which the Administration's responsibilities for basic research can be made distinct from its other responsibilities. It is hard to see how this can be accomplished unless there is a strong coordinating body which includes the directors of the National Science Foundation and of the NIH, the research directors of the executive agencies at present supporting basic research and a strong representation from the universities.

It is also clear that the Administration is desperately in need of some means of making sense of its hitherto incoherent policies on technology. Like other governments, it has set its heart on finding means by which public expenditure can be used to stimulate industrial innovation in the civil field and to yield results of social benefit. Like other governments, it has so far failed. The difficulty has been that governments, advised by thoughtless people such as Mr William H. Magruder, have specified grand objectives and have then hoped that technologists will wave some magic wand to make cities decent, surface transport innocuous and earthquakes go away. The truth is, however, that in technology as in science, progress depends on the accumulation of small innovations whose ultimate uses cannot easily be predicted. What the American government needs is a mechanism for using the many public laboratories in the United States for maintaining a detailed watch on developments in their field of competence, for seizing new opportunities and for spending what money the government can afford. The Federal Council on Science and Technology, not a very useful body, could well be replaced by some device for controlling these activities and making sure they are sensibly directed. But here again, the Administration will have to acknowledge that it cannot make policy on its own.

Trouble in the Loch

WHATEVER the final outcome of the application by the Gas Council and the Total Oil Company to build a gas terminal at Crimond airport in Scotland, there are lessons to be learned—and which should have been learned a long time ago. The first thing to be said is that the application, which involves the construction of a gas processing plant on the shores of Loch Strathbeg, concerns a scientifically important site with important physical and aquatic features (see page 80 and *Nature*, **240**, 435; 1972). The second is that the terminal must be built somewhere. Britain needs North Sea gas and Scotland needs the jobs. That said, however, questions remain about the choice of site and, more important, about the way in which the provisional choice has been made public.

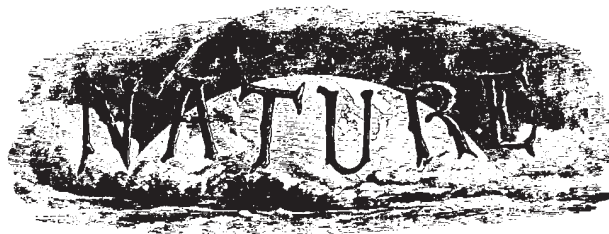
The site is clearly important. The Nature Conservancy says so, the Royal Society for the Protection of Birds says so and the opponents of the scheme, including Professor V. C. Wynne-Edwards, former chairman of the Natural Environment Research Council, are men of calibre, unlikely to get excited about nothing. Yet in spite of the importance of the site—and it would be naive to pretend

that the Gas Council was unaware of its value—the first the Nature Conservancy officially heard of the terminal was when the Aberdeen County Council advertised on December 5 for objections to reach it within a fortnight. The conservancy, and other interested bodies, were therefore given two weeks to produce a scientific defence of a scientific site. Two months might have been more fitting.

Might not the Gas Council and Total have been somewhat freer with information about the scheme? The council says that the application was only for outline planning permission but this is a nonsense. How is it possible to tell what damage will be done until full details of the scheme are known and the likely effect on the loch accurately assessed? Similarly, Total's refusal to comment on the proposal before it went to the planning committee does not exactly encourage faith in the assumption that all other sites have been examined and that full account has been taken of the effect of the terminal on the loch. As things have turned out, Aberdeen County Council has had the good sense to postpone a decision on planning permission until fuller details are available and until the conservationists' objections can be better heard. The Gas Council and Total have this week been meeting the county council's countryside committee. Yet both the sponsors of the scheme could have saved themselves the trouble of ducking the mud thrown at them since the scheme was announced if only they had had the sense to consult other interested parties in advance.

As things are, if Crimond proves to be the only practicable site, the Gas Council and Total will have to live with the considerable hostility engendered by their handling of the matter. If some other site is finally chosen, they will somehow have to moderate the considerable distrust of conservationists in the industry's ability to consider interests other than its own. A little consultation early in the day could have saved the Gas Council and Total considerable time and effort (particularly if an inquiry holds up the application for months), and would also have avoided yet another head-on clash between conservationists and developers. Surely that is not too much to ask.

100 Years Ago



THE Octopus in the Brighton Aquarium met with a sad fate on Jan. 7. Finding himself uncomfortable in a tank where he had been newly placed by the curator, he came out, in an unguarded moment, of the house of living oysters he had collected as a shelter round him. In this tank were several large specimens of spotted dog-fish. One of these fish, with the true 'cuteness' of a sea-uug, immediately pounced upon the unsuspecting octopus, and swallowed him.—Another novelty has been introduced into the Brighton Aquarium, viz, the apparatus for carrying on salmon and trout hatching. The trout from the Trent are thriving splendidly.

From *Nature*, 7, 209, January 16, 1873.

OLD WORLD

European Science Cut Four Ways

A FAR-REACHING reorganization of responsibilities for science and technology within the European Community has followed on the addition of Britain, Denmark and Ireland to the community.

The wide-ranging responsibilities held by Mr Altiero Spinelli until Sunday's meeting of the new commission have now been parcelled out among four commissioners. Mr Spinelli had been in charge of what were labelled industrial, technological and scientific affairs but also included a small education unit. Now Mr Spinelli is responsible only for industrial and technological affairs, and Dr Ralf Dahrendorf of West Germany, formerly commissioner for external relations and trade, has taken over scientific research matters and education. Energy policy, and those aspects of Euratom's work that affect energy policy, formerly the responsibility of Mr Wilhelm Haferkamp, now fall under Mr Henri Simonet, the Belgian commissioner, while Mr Carlo Scarascia Mugnozza, the second Italian commissioner, is to have a wide-ranging brief including environmental matters, transport and consumer interests as well as public information.

These are early days, and the full implication of the changes will not become apparent at least until the directors-general and the directors have been appointed, but it is thought that Mr Mugnozza could build for himself a powerful brief in the field loosely termed the "quality of life". But it is into Dr Ralf Dahrendorf's hands that the bulk of scientific matters now falls. He is to have responsibility for the much-troubled Joint Research Centre that is the nub of Euratom, and his first problem will come to a head on January 18, when the commission's plans for reducing Euratom's size come up again for consideration in Brussels.

The commission is acutely aware of Euratom's failings, and there is also a feeling that the JRC is too big for the job that is left for it. Fundamental research on nuclear power has suffered from the way in which national interests have set off independently in producing nuclear power plants, and the JRC, with 2,000 staff and four centres in Italy, the Netherlands, Germany and Belgium, has not enough work to keep itself occupied. Plans to move the JRC into general contract research for industry on the now familiar lines of the UKAEA's Harwell establishment fell down because Euratom's founding

treaty permits virtually nothing to be done within Euratom except nuclear research.

The community is unwilling to continue funding such an expensive enterprise (\$44 million in 1972), but running it down is difficult as its scientists are European civil servants. Nevertheless the commission has produced plans to close the Dutch centre at Petten which concentrates on materials testing and significantly to reduce the numbers at the Italian centre at Ispra—by far the largest of the four centres, currently employing about 1,200 people. The objective is to reduce the total staff to about 1,600. This may not be radical enough. At a meeting in December, it became apparent that the French and German governments want the staff at the JRC reduced still further to about 1,200, and the fear in the commission is that unless a compromise is reached, the JRC could be cut back so heavily that it will be unable to fulfil any useful function. Sorting out this knotty problem, not to mention the complicated financing of the centre, is now on Dr Dahrendorf's plate, although Mr Spinelli will in fact be battling for what are essentially his proposals at the January meeting.

Dr Dahrendorf is also to have the task of trying to "harmonize"—a favourite European word—the various qualifications and degrees that are earned around Europe.

The separation of science and research from technology and industry—technology apparently includes research on such matters as aerospace and computers—could improve the chances for the European Science Foundation, and could ensure a commitment to fundamental research stronger than might have been the case within a brief including technology and industry. On the face of things, Dr Dahrendorf's responsibilities for science would appear not dissimilar to the scope of activities of the British Government's Department of Education and Science.

METEOROLOGY

Cloud Seeding Attacked

IN a sweeping attack on ill-conceived attempts at weather modification, Dr B. J. Mason, Director-General of the UK Meteorological Office, has warned that the reputation of meteorology could be severely damaged and that the science could become embroiled in inter-

CRIMOND TERMINAL

Consultation Begins

THE Gas Council and Total Oil Marine are to hold talks with the objectors to their scheme to build a gas terminal at a site of scientific importance.

Following a meeting with Aberdeen County Council's countryside committee this week, it was agreed that representatives of the industry will provide answers to specific queries about the application put to it by the committee, and that they will meet representatives of the scheme's opponents including the Environmental Liaison Group that has been set up around the university.

The proposal to build a gas terminal at Crimond airfield above Loch Strathbeg first came to light in December when the Gas Council and Total Oil Marine applied for planning permission. Fourteen days were given for objections to the scheme to reach the planning committee. In that time more than 170 objections, some of them protest petitions, reached the planning committee

which decided to postpone a decision until more was known of the proposal.

Loch Strathbeg is classified as a site of scientific importance on wildlife, physical and limnological grounds, and the scheme's opponents are eager that the site should be preserved because of its international importance.

The planning committee is due to meet next on January 22, by which time the Gas Council and Total will have met the objectors to see if "safeguards can be evolved to allow the planning proposal to go ahead", according to Mr John Russell, Aberdeen's county clerk.

The key to the problem, it appears, is that the submarine relief and the nature of the sediments off the north-east coast of Scotland are among the crucial factors in deciding where the pipeline is to come ashore. One off-shore survey was completed last summer, but other surveys are still in progress and as far as Professor Kenneth Walton, professor of geography at the University of Aberdeen, is concerned "there is still not sufficient information available on which to base a firm conclusion".

national politics if such attempts continue.

In a speech to the International Cloud Physics Conference, reported in *Weather*, Dr Mason says that, "with some notable exceptions", attempts at weather modification "have generally failed to conform to the accepted principles and standards of scientific experiment and analysis, and are therefore incapable of providing objective answers to such questions as to whether, in what circumstances, and to what extent it is possible to modify precipitation by artificial seeding. . . . Politicians and entrepreneurs are initiating and conducting major weather modification projects without the benefit of proper scientific direction, advice and criticism, and this may have serious repercussions on the reputation of meteorology as a science and profession".

Dr Mason went on to complain "that such operations are being promoted on the assumption that the basic assumptions and techniques are already proven and that the remaining problems are largely of an engineering or logistic nature". But in fact "the protagonists of this approach are continuing to use the same inadequate concepts and techniques that have failed to provide convincing answers during the past twenty years".

"We hardly begin to understand the processes that control the intensity, durability and distribution of precipitation as witnessed by our evident inability to predict these quantities within fifty per cent, even for short periods—shorter than the lifetime of the storm. . . . This leads me to challenge the thesis that weather modification is the main practical justification for cloud physics research and to suggest that, taken world-wide, the potential economic and social benefits of accurate prediction of precipitation outweigh those of modification for the foreseeable future".

Dr Mason went on to say that while, in emergencies, weather modification might be attempted in the hope of ending severe droughts, a sharp distinction should be drawn between such operations and carefully designed experiments "that have some chance of producing answers to properly formulated scientific questions".

The international implications of weather modification also alarm Dr Mason. Suggestions that the World Meteorological Organization should advise the developing world on cloud seeding should be treated with caution. Such advice should not be given until thorough on-the-spot investigations of local conditions had been completed, and even then "an advanced country should consider very carefully the wisdom of urging on a developing country largely unproven techniques".

According to Dr Mason, Project Whitetop, one of the few well designed weather modification programmes, in which Missouri clouds have been seeded with silver iodide for a five-year period, suggests that rainfall actually decreased between 40 and 60 per cent in the 100 miles downwind of seeding, although there may have been some increase 180 miles downwind.

Dr Mason is also concerned that if weather modification does become feasible on a large scale, "an ill-judged attempt by any nation to carry out such a project without international agreement would, almost certainly, plunge meteorology into the cockpit of international politics, controversy and disension". The effect on programmes such as the World Weather Watch and the Global Atmospheric Research Programme could be disastrous, Dr Mason says.

His solution is that meteorologists should, through learned societies, scan weather modification programmes carefully and "grant scientific recognition and approval only to those that fulfil rather strict scientific criteria, have scientific direction and are open to inspection and evaluation by independent scientists". Projects that are not vetted should not be eligible for presentation at scientific meetings or for publication in scientific journals. Dr Mason admitted this week that such a proposal might be labelled as censorship, but he argued that the situation is potentially so serious that the profession must safeguard its scientific integrity.

SOVIET SCIENCE

Vavilov Commemorated

from our Soviet Correspondent

THE Soviet Ministry of Communications has issued a special commemorative envelope to mark the thirtieth anniversary of the death of Academician Nikolai Ivanovich Vavilov, "the Soviet botanist, geneticist and selectionist", thus marking a further stage in the rehabilitation of the leader of the anti-Lysenko conflict of the 1930s.

Vavilov, whose outstanding services to Soviet agriculture in crop breeding and genetic selection are beyond dispute, was arrested in July 1941 on a number of charges ranging from "sabotage in agriculture" to "spying for England" and "belonging to a rightist conspiracy". His real offence, however, was his opposition to Stalin's sponsorship of the theories of Lysenko. Vavilov was sentenced to death and, although the sentence was later commuted to ten years imprisonment, he died in Saratov prison on January 26, 1943.

Vavilov was posthumously rehabilitated by the Soviet Supreme Court on September 2, 1955. A week later, the

praesidium of the Academy of Sciences restored his name to the register of deceased members. Since then the rehabilitation process has gone slowly and tacitly forward. In 1960 a Vavilov memorial volume was published. Articles praising his work have appeared from time to time in the Soviet press. November 26, 1971, the 84th anniversary of his birth, was marked by the unveiling of a memorial plaque on the wall of the Institute of Genetics in Moscow.

SPACE RESEARCH

Astrophysics Unit Moves

THE Science Research Council's Astrophysics Research Unit has been transferred to the council's Radio and Space Research Station at Slough. This transfer, effective from January 1, adds a strong astrophysical side to the support work already carried out by the station.

RSRS is the SRC's chief support centre for university space research, and currently provides such services as prediction satellite orbits, environmental testing of space experiments and provision of data centres for rocket, satellite, ionospheric and solar data. The station also provides support for radio astronomers, and the SRC's Space Research Management Unit which plans rocket and satellite programmes is there.

This supportive role for Slough emerged from an SRC panel report on the station in May 1971, and the addition of the astrophysical unit means that most SRC support of university space work can now come from one centre.

The decision to move the unit ensures its continuity which was very much in question following an examination, in 1968, of the work of the United Kingdom Atomic Energy Authority's Culham laboratory of which the unit was a part. At the time it was suggested that the unit might be disbanded, but the SRC took control and its work on solar physics, laboratory astrophysics and ultra-violet astronomy continued, with the unit still at Culham.

A large part of the unit's work already involves collaboration with university work, so its new role will not be a radical change of direction. Although the unit is now under the control of Dr J. A. Saxton, the director of RSRS, and is now one of the station's divisions rather than an organization in its own right, the unit's staff of forty will not be moving to Slough for some time. Extra accommodation will have to be provided before a physical move can actually take place.

Dr R. Wilson, who was director of the astrophysics unit, has now moved to University College, London, to take the post of Perren Professor of Astronomy.

NEW WORLD

David Resigns, Changes in the Offing

by our Washington Correspondent

DR EDWARD E. DAVID, JUN., sprang a surprise last week by resigning as Director of the Office of Science and Technology and Science Adviser to the President. He told his staff soon after the new year's holiday and later that day flew to Chicago to take up his new job as executive Vice-President of Gould Inc., a manufacturer of electrical and electronic equipment. No successor has yet been announced and, indeed, the big question is whether or not David will be replaced, for his departure has intensified speculation that the science policy machinery in the White House may be drastically reorganized.

David's resignation was accompanied by the expressions of regret and thanks from the President which are usual on such occasions, and he has said himself that he is not leaving because of any dispute or with any sense of disappointment. Indeed, there is nothing extraordinary in his serving only two years of what is, on paper, a six year term of office—there have been six science advisers since the job was created.

David's departure has nevertheless prompted speculation that he has quit because his advice too often fell on deaf ears. This suggestion was played down by some White House officials last week, but others did not deny the possibility. One ominous possibility is that his departure is linked with the negotiations on the science budget for 1974, now almost complete.

It is generally accepted that this will be tightly squeezed. A recent study by the Brookings Institution suggested, for example, that in order to meet his goal of keeping federal expenditure below \$250,000 million, President Nixon must cut some \$7,000 million from present expenditures. Although science and technology accounts for only a relatively small part of the budget, it unfortunately falls into the category which is cuttable—all but about \$72,000 million of federal expenditures are earmarked for items such as social security and pensions which cannot be reduced.

Whatever the reasons for David's departure, it cannot be denied that it was kept a closely-guarded secret. One White House official said last week, for example, that "although some of us knew he was thinking of leaving, few of us thought he would do it". The news of his impending departure had also not reached the President's Science

Advisory Committee (PSAC), of which David was chairman.

An electronics engineer who spent 20 years working for Bell Laboratories Inc. before joining the White House in 1970, David was Science Adviser to the President during a particularly difficult time. He was appointed when expenditures on science and technology were declining, and lived through some tricky political negotiations over the federal government's plans to stimulate industrial research and development and to bring science and technology to bear on domestic problems. Throughout his tenure of office, he sensibly kept "a low profile", which makes it difficult for outsiders to judge his effectiveness, but he is widely respected in the scientific community. During the past two years, expenditures on science and technology have even shown a modest increase.

David's most difficult time was in 1971, when Mr William Magruder was brought into the White House to take charge of what was called the "New Technology Opportunities Program" (NTOP). The idea was to find ways to stimulate industrial research and development and to plan new opportunities for using science and technology to solve problems such as pollution control and urban decay. Magruder's appointment was viewed as a slap in the face for OST, and his approach, as characterized by Dr Lewis Branscomb in a speech given at the AAAS last month, was to argue for large federally funded projects. The outcome could be seen in the President's Message on Science and Technology, which outlined a policy base, but included few big projects. David emerged relatively unscathed from that process, and the OST's political influence was, if anything, increased.

Now that David has departed, what lies in store for the OST and PSAC? The first thing to be said is that the post of Science Adviser to the President and PSAC itself were both set up by the President in 1957, and the Office of Science and Technology was established in 1962 by an Executive Reorganization Plan. None of them, therefore, has a Congressional charter, and all could be scrapped by the President without the consent of Congress. It would thus seem logical that if changes are in store for the White House science policy machinery, this would be a good opportunity. But, as one White House official remarked last week, "that assumes that

we do things logically, which is a big assumption".

The most common view appears to be that OST is likely to be tied in more closely with the Office of Management and Budget, chiefly on the grounds that such an arrangement would allow it to give more direct support to the Nixon Administration's general philosophy that science and technology must be supported on the basis of their possible payoffs in economic growth and socially useful innovations. Relevance is the watchword. Although OST has always given advice on the budget as it relates to science and technology, its relationship with OMB has been obscure.

As for PSAC, no action had been taken at the end of last week on its pro-forma resignations which the members submitted after the election, but two recent events suggest that President Nixon does not think much of the committee. First, it took seven months to fill three vacancies on PSAC—the terms of office of three members expired on December 31, 1971, and they were not replaced until August 1972. Second, the Administration is embarrassed by a PSAC report on the SST. Called the Garwin Report, it was critical of the SST project at a time when the Administration was backing the project, and although it was meant to be secret, its recommendation that the government should withdraw support from the SST prototype leaked out. Eventually, after a lengthy court battle, the OST released the report. If President Nixon does intend to scrap or modify PSAC, however, his plans have not been disclosed to the committee's members.

OST and PSAC are not alone in their anxiety about the future. At the end of last week, four other key scientific posts were unfilled. No successor to Dr James Schlesinger, who is being moved from the chairmanship of the AEC to become Director of the CIA, had been announced. There are also vacancies at the head of NIH, at the Department of Health, Education and Welfare, where Dr Merlin K. DuVal recently resigned as Assistant Secretary for Health, and in the Department of Defense, where Dr John S. Foster Jun. is resigning as Director of Defense, Research and Engineering.

Whatever arrangements the Administration makes, it is likely to find itself embroiled with Congress on several fronts, not least on the question of whether Congress or the White House

holds the pursestrings. With Democratic control of the Senate strengthened, with the past year's record of occasions on which the White House has withheld funds appropriated by Congress and under the stimulus of protest at the renewed bombing of Vietnam, many members of Congress are up in arms about the constitutionality of the Administration's relationship with the Capitol.

Last week, for example, seventeen senators, including the chairmen of thirteen committees, majority leader Mike Mansfield and majority whip Robert Byrd joined with the state of Missouri in a suit against the Administration which alleges that the withholding of highway trust funds last year was illegal. A federal judge ruled in favour of the state last year, but the Administration has appealed. And a bill has now been introduced by Senator Sam Ervin, chairman of the Senate Government Operations Committee, which would require the President to inform Congress thirty days before any funds are impounded, thus allowing his decision to be overridden.

The debate on the pursestrings is

important to science and technology because the Administration is expected to withhold between \$6,000 and \$10,000 million of the money appropriated by Congress for projects in the 1973 fiscal year, now more than half way through (see *Nature*, 240, 247; 1972).

Congress and the Administration are also likely to be at odds over a specific piece of legislation involving science policy—Senator Kennedy's National Science Policy and Priorities Act. Passed last year by the Senate by a vote of 70-8, the bill failed to emerge from the House Committee on Science and Astronautics before the 92nd Congress dispersed. It was, however, introduced again by Kennedy last week, and stands a good chance of passing through the Congressional mill this session. President Nixon would, however, be certain to veto the bill because it calls not only for \$1,800 million to be spent over three years on research and development directed towards domestic problems and on retraining unemployed scientists but also for expenditures on research and development to increase faster than the gross national product.

In the more immediate future, Congress must conjure up an appropriations bill for the Department of Health, Education and Welfare which is acceptable to President Nixon. Although the 1973 fiscal year started on July 1, 1972, NIH and the other agencies in HEW are having to make do with the same level of funding they received last year, because Nixon twice vetoed 1973 HEW appropriations which he considered excessive. Congress can be fairly certain, however, that the Administration will impound any funds which are added on to its 1973 budget request for HEW. The fight for control of the pursestrings will thus be followed with close attention.

NASA

HEAO Shelved

by our Washington Correspondent

THE National Aeronautics and Space Administration announced last week that it has halted work on the High Energy Astronomy Observatory (HEAO) and on nuclear propulsion, that it is phasing out research and development on communications satellites, and sharply curtailing work on nuclear power. These casualties are direct consequences of President Nixon's campaign to hold federal spending down to \$250,000 million this fiscal year, and they indicate that NASA will be particularly hard hit by budget cuts.

Work on HEAO has been suspended for at least a year, while NASA officials study "ways to meet some of HEAO's objectives at lower costs", which is being

interpreted to mean that the satellite has been shelved, at least until the space shuttle is available to put it into orbit. The news has been greeted with bitterness by astronomers, who regard it as perhaps the most important satellite planned by NASA—it has consistently been given the highest priority by the Space Science Board of the National Academy of Sciences, for example.

NASA officials decided to apply the knife to HEAO rather than to other expensive projects for two reasons. Not much money will be wasted if it is scrapped, because the project is only just getting under way, and it is not tied to a launch date because it will be placed into Earth orbit. Only about \$18 million have been spent out of a total estimated cost of \$250 million for the project. Scrapping HEAO will, however, severely stunt the growth of high energy astronomy because although rockets, balloons and small satellites can be used for high energy experiments, heavy instrumentation is required for high sensitivity and resolution. So far, two high energy astronomy satellites have been launched—an X-ray astronomy satellite and a gamma ray astronomy satellite—and another satellite to study soft X-rays is scheduled for launch in 1975. Astronomers are now hoping that some of the experiments planned for HEAO can be flown on smaller and less expensive satellites.

As for communications satellites, NASA officials have decided that they should now be developed by industry. NASA has provided the initial costs of research and development on communications satellites, and the agency will launch the next in the series, ATS-F, in 1974. All work on ATS-G has, however, been stopped.

The decision to scrap research and development on nuclear propulsion and to curtail work on nuclear power systems was taken because "all prospective applications are in the very distant future". NASA in fact decided a year ago to scrap its development of a large nuclear rocket, and to concentrate on a smaller system, but that decision was greeted with some distaste, especially in Congress (see *Nature*, 235, 415; 1972). The decision to scrap all development of nuclear propulsion will, however, be more widely criticized because planetary scientists were hoping that nuclear engines would be capable of taking spacecraft to the outermost planets in the late 1980s. Without the Grand Tour and nuclear rockets, the outer planets are likely to be out of reach at least this century.

Although the cuts announced last week were made to save money in the present fiscal year, they provide some pointers to NASA's plans for 1974. The Viking mission to make a soft landing on Mars is likely to go ahead, for

All Aboard

ONE of the first acts of the 93rd Congress was to appoint Senator Clifford Case to the board of the Office of Technology Assessment and to confirm that Senator Edward M. Kennedy will be the board's first chairman. In addition, Olin Teague of Texas, the new chairman of the House Committee on Science and Astronautics, is expected to be given the place vacated by the departure from Congress of Earle Cabell. Senator Case, a Liberal Republican from New Jersey, replaces Senator Gordon Allott. Allott and Cabell both lost their seats in Congress in the November elections.

The new board is, however, having some difficulty in getting together—all twelve members must be present for the first meeting so that a quorum can be fixed—and the first session is unlikely to be before the end of the month. And it will be April at least before the office gets under way, for the director and staff cannot be appointed until funds are made available by the appropriations committees. Although the bill which established the office authorized \$5 million to start it up, the money will not be available until a supplemental appropriations bill has gone through Congress, probably lumped in with other supplemental appropriations in the spring.

example, otherwise it would also have been scrapped now to make the maximum savings. Similarly, hopes are high that the Mariner mission to Jupiter and Saturn will be saved from the axe. But as late as the end of last week, NASA officials were still engaged in hard bargaining with the Office of Management and Budget. At least one project is being dealt with lightly: the space shuttle has had only minor cuts in manpower build-up, and it will certainly go ahead.

SENATE COMMITTEE

No Fight for Space

THE Senate Democratic leadership sprung a surprise last week by appointing Senator Frank E. Moss of Utah to the chairmanship of the Committee on Aeronautical and Space Sciences. Moss, a widely respected Senator who was first elected in 1958, has never been a member of the committee. Although he has consistently voted in favour of the space programme, he has not shown an unusual amount of interest in space affairs. His appointment came about because none of the members of the committee who were in line for the chairmanship wanted the job.

The chairmanship of the space committee, vacant because of the retirement last year of Senator Clinton P. Anderson, a founder member of the committee, would normally have been passed to Senator Warren Magnuson. Understandably, however, Magnuson preferred to hang on to the chairmanship of the more powerful Commerce Committee, and the offer went to Senator Stuart Symington. But he said last week that his commitments on the Armed Services Committee, the Foreign Relations Committee and the Joint Committee on Atomic Energy are more than enough to keep him occupied. The next in line, Senator Howard Cannon of Nevada, has chosen to hang on to his chairmanship of the Rules Committee. Their reluctance to take on the chairmanship of the space committee is a good measure of the decline in the committee's importance.

NIH

Fallout from Marston

by our Washington Correspondent
THE forced resignation of Dr Robert Q. Marston as Director of the National Institutes of Health (see *Nature*, 240, 437; 1972) has sparked off a wave of protests from scientists at NIH itself. The reasons why President Nixon accepted Dr Marston's *pro forma* resignation have not yet been spelled out and a successor has not yet been nominated, but it is widely felt that Marston's resignation was brought about for political reasons, and that he will be replaced by

someone closer to the Administration. One view is that Dr Marston has paid for not having fought hard enough for the Administration's cancer bill, which would effectively have taken cancer research outside NIH.

The Inter-Assembly Council of the Assemblies of Scientists, which represents professional scientists at NIH, wrote last week to the President to complain that this is the first time that a director of the NIH has been made to resign after a presidential election, to urge that Dr Marston has done a good job and to hope that his successor will be a person of stature in research, not merely an administrator.

AAAS

Genetics, Good or Bad

by our Special Correspondent

THE Youth Council of the AAAS provided some spirited discussion at its December meeting of the legal and

sociological implications of one branch of science by a symposium on genetics, man and society. Organized jointly with the Yale University Task Force on Genetics and Reproduction, the meeting aired many diverse points of view, not only from the expert members of four inter-disciplinary panels but also from the lively audience.

About half the allotted day was devoted to talk of screening and genetic counselling programmes. Obvious though the advantages of being able to predict before birth that a child will be afflicted with a crippling disease such as muscular dystrophy may seem, there is room for heated argument. For example, the recently introduced requirements of some states and municipalities that members of the black population should be screened for sickle cell anaemia have sparked off great controversy which was reflected in the opinions expressed at the symposium. Thus Dr H. A. Bender, a biologist from the University of Notre Dame, suggested that critics of this programme are foolishly asking for the right not to know about their genetic problems. This, he said, may have incalculable costs but consequences such as harsher insurance rates or diminished opportunities of employment for people whose sickle-cell trait has been detected stem from the failure of scientists to educate the population properly. By contrast, Dr I. Ladimer, a physician and lawyer from Mount Sinai School of Medicine, suggested that the sickle cell anaemia screening programme is unethical so long as there is no really effective therapy available.

On *in vitro* fertilization and the subsequent culture of human oocytes, one view at this meeting was that the technique may be valuable for women who are infertile because their oviducts are blocked. Dr B. G. Brackett, who is engaged in this work at the University of Pennsylvania, felt that in spite of the present technical barriers, it will eventually become possible to implant fertilized ova in such women.

On the question of legislative measures to regulate the use of genetic knowledge, Mr H. Jasper, a legislative assistant to Senator W. F. Mondale, pointed out that probably only four or five senators and even fewer representatives know more about genetics than the lay public. He advocated action now to establish a framework for policy-making, rather than to wait until a disaster prompts the hasty passage of specific and poor legislation. The former course is implicit in Senator Mondale's proposal for a public advisory commission to make a two-year study of the ethical aspects of biology included in the bill he steered through the Senate last year but which eventually died in a House committee room.

Change at the Top

by our Washington Correspondent

RESIGNATIONS and appointments for natural causes still take place in Washington. Dr Rocco A. Petrone, Director of the Apollo Programme, will become director of the Marshall Spaceflight Center in succession to Dr Eberhard Rees, who retires on January 19, and George Vineyard jun. has been chosen to succeed Dr Maurice Goldhaber as director of the Brookhaven National Laboratory. Dr Goldhaber is returning to full-time research.

The announcement of Rees's retirement and Petrone's appointment was neatly stage-managed to coincide with the end of the Apollo programme, in which both played a significant part. Petrone had directed the programme since September 1969, while the Marshall Spaceflight Center has been responsible for the Saturn Launch vehicle and the development of the Lunar Rover. Rees was deputy director of the center from 1960 until he succeeded Dr Werner Von Braun in 1970.

The new chief at Brookhaven has been deputy director for the past five years. A solid-state physicist, he takes on his new job when Brookhaven is diversifying into such fields as the environmental effects of various types of energy generation. But his chief headache will be the continuing shortage of funds for high-energy physics, particularly in relation to Brookhaven's proposal for a new colliding-beam device.

NEWS AND VIEWS

How Useful is Earthquake Prediction ?

FOR many years seismologists have been measuring the velocity of compressional (P) and shear (S) waves in rocks and studying the relationship between these velocities and the elastic moduli and density. These parameters can change with changes in the applied stress; before an earthquake occurs the stress in the focal region increases, and if this increase can be monitored it may lead to a method for predicting the onset of the elastic failure responsible for earthquakes. Attempts to monitor P and S velocities in the past, using explosive charges over a measured line, never came to anything, and interest in this type of experiment waned. That was until 1969, when Semyenov published the results of an experiment in the seismic area of Garm in the Soviet Union (*Izv. Phys. Sol. Earth*, 4, 245; 1969). Semyenov showed that, before an earthquake with a value of m_b of the order 4 or more, there is a significant variation in the ratio of the P and S velocities. He reported observations of microearthquakes in the Garm region which showed that the ratio v_p/v_s remained fairly constant at about 1.77 ± 0.01 for considerable periods of time before v_p/v_s began to decrease to a minimum value of less than 1.70 followed by a sharp increase before a larger earthquake.

On page 101 of this issue of *Nature* a significant confirmation of these Soviet results is reported by Aggarwal and his colleagues of the Lamont-Doherty Geological Observatory. The significance of their work is that they have repeated the Soviet experiment in a completely different region of the Earth, namely the Blue Mountain Lake area of New York State, and have obtained very similar results. They, too, used microearthquakes as an energy source to monitor and establish the norm of the v_p/v_s ratio. In the Blue Mountain Lake area, they deployed a network of high gain seismograph stations, which were sufficiently close to obtain hypocentre information with adequate precision. Although no seismograms are illustrated in the article whereby the reader can judge how precisely the onsets of the P and S waves can be measured, the authors affirm that these arrivals are very clear and impulsive and can be read consistently to an accuracy of 0.01 s. Certainly the data show very little scatter, and as in Garm the ratio v_p/v_s decreases to a minimum and increases sharply before an earthquake.

Why should the ratio v_p/v_s vary in this way when an earthquake is about to occur? Nur has attempted to explain the Soviet observations by suggesting (*Bull. Seism. Soc. Amer.*, 62, 5; 1972) that the decrease in the ratio may be the result of dilatancy, that is to say decreased volumetric strain, of rocks in the focal region as a consequence of increased stress. The sharp increase in the ratio just before the earthquake may, he suggested, be caused by the flow of ground water into cracks formed by dilatancy. Increasing the pore pressure in this way weakens the rocks. A similar interpretation is presented by Aggarwal *et al.* in their article.

This hypothesis is also used to explain the increased seismic activity observed in Colorado when effluent fluids were pumped into deep boreholes. The increased seismicity around new dam and reservoir sites is similarly explained; further development of the idea should come from seismographs now commonly installed around new reservoir sites. At these sites the experiment can be carried out at all stages of construction before and after the reservoir is filled.

What does this technique contribute to the art of earthquake prediction? For the present it would seem very little, for it remains to be seen whether or not the observations are caused by shallow and superficial phenomena. The observed variation in the velocity ratio would seem to be very local and is a precursor to very small and particularly shallow earthquakes (about 3.5 km). The costs of the comprehensive network of stations which would be required for monitoring, their operation and the continuous processing of the data are far from trivial. Other observations of geophysical changes preceding an earthquake, such as small changes in the electrical conductivity of the rocks and variations in radon exhalation as the rock is stressed, are similarly localized and would suffer from similar logistic problems if they were to be applied in an operational sense.

It is also worth drawing attention in this context to the tendency for the more scientifically interesting problems associated with earthquake prediction and control to obscure the fact that the application of well-known civil engineering methods would mitigate the loss of property and life in cities, and for the more useful, if less glamorous, projects concerned with the problem of well-insulated, low-cost housing for villages in seismic areas to be starved of funds. There is little to be gained by predicting earthquakes, such as the one which recently hit Managua, if afterwards there is no city to which the temporarily evacuated inhabitants may return.—From a Correspondent.

Synthesizing Diamonds

PRECIOUS stones must satisfy two criteria—they have to sparkle and they have to be hard. Diamond, being the hardest material known and possessing a high refractive index and good dispersion, obviously qualifies; but in addition it has one property of even greater value for the world at large—it is not found in significant quantities in the United States.

For this reason the decision was taken in 1951 to "mount a major new assault on the old and bloody battlefield" of diamond synthesis; the US General Electric

Company announced success on February 15, 1955, and in 1960 Dr Guy Suits described this operation in an article entitled "The Synthesis of Diamond—A Case History in Modern Science", written, as he explained, because "traditionally, the history of political events and warfare between nations is recorded in minute detail . . . But we have not yet established a tradition of recording in historical detail the events of science and technology, which are increasingly playing a decisive role in human welfare and the affairs of nations". The point is illustrated by his observation that "the ultrahigh pressure, high temperature process for forming diamonds, which at first strained existing high pressure technique almost to its limits, has in only a few years become a routine production process which has turned out millions of carats of diamonds. As a result, this country [the United States] has been relieved of a critical dependence on industrial diamonds from the Congo".

The diamond project was not cheap (I think I first heard the word "megabuck" in connexion with diamond synthesis). One reason, as Dr Suits explains, was that so much exploratory work had to be done, and blind alleys and detours absorbed almost as much human energy as the successful portion of the project. One had to be very careful about jumping to conclusions; as one of the team wrote in his notebook: "In each sample I found many absolutely perfect octahedrons . . . They certainly look like the real thing . . .", but they were subsequently found to be chromite, originating in the chromium of the stainless steel reaction chamber. Nor did the development of apparatus always proceed smoothly; it was soon found that gaskets made from the pipestone of the American Indians, as used in the pioneer high-pressure experiments of Bridgman (and selected for him by one particular Indian in the best traditions of alchemy), had to be abandoned in favour of the much less romantic but far more satisfactory material, pyrophyllite. Above all, "the measurement of pressure and temperature in the original cell, and all subsequent cells, turned out to be a continuing source of difficulty and uncertainty".

Neither was it easy to learn from natural diamond. In 1952 attempts were made to establish the graphite-diamond equilibrium curve experimentally by graphitizing diamond, but "unexpectedly, determination of the onset of graphitization proved to be difficult. Different diamonds behaved differently. Some showed surface blackening, some became smoky, and some remained clear and resisted graphitization even at relatively high temperatures". These remarks emphasize that in diamond research the concept of the "average diamond" is about as useful as that of the "average Briton" for all but the most trivial investigations.

But General Electric persevered with the synthesis experiments, and eventually found that iron sulphide in a graphite tube with tantalum end-disks produced diamonds; in fact many metals could be used, provided that the temperature was high enough to melt the metal when saturated with carbon. Much of their work was summarized in a communication in *Nature* (184, 1094; 1959), and in 1960 Dr Suits could write, "With a great range of experimental conditions available through a choice of temperature, pressure, catalyst-solvent and time, it is not surprising that the size, crystal habit, colour, clarity and other properties of man-made diamonds are subject to a significant degree of control. Diamond can

be grown for specific industrial applications. . .".

If the Congo suffered as a result of this work, geophysics did not. The impact of this achievement can be seen very clearly in the opening pages of the Annual Reports of the Director of the Geophysical Laboratory in Washington. Already in 1956–57 the report says that "when equipment currently under development is operable, this laboratory will be able to participate very actively in advancing one of man's great frontiers—high pressure studies". In 1957–58 it points out that "knowledge of the mineral constitution of the upper mantle and of the deeper parts of the Earth's crust is fundamental to progress in many fields of geophysics and petrology . . . Interpretation of seismic discontinuities and working out the origins of rocks such as eclogites, kimberlites and granulites are only a few of the problems whose solutions depend . . . on our knowledge of mineral equilibria at greath depth. Progress in this field depends directly on our ability to duplicate in the laboratory the conditions of high pressure and temperature that exist at depth in the Earth". In 1959–60 the report actually begins: "Results of fundamental research frequently are universally applicable. Knowledge of phase equilibria in silica and sulphide systems gained at the Geophysical Laboratory has repeatedly been applied to mineral association in rocks from all the continents and to meteorites. Application of our work is likely to be extended further, since personnel of this laboratory are currently participating in the planning phases of two new frontiers of earth and planetary science—the space program and the Mohole project". In 1965–66 one reads that "progress in science is often contingent on the development of new apparatus. This is especially true in a field such as high pressure research in which workers are continually seeking means of studying phenomena under ever more intensive conditions".

While the geologists were revelling in the new possibilities opened up by the advent of successful high pressure technology, the scientists at General Electric had not been idle. They succeeded in growing gem diamonds in a variety of colours with shapes so reminiscent of the well known emerald cut that acceptable gems could be obtained from them without significant loss of weight on cutting—a very important bonus when the weight loss involved in faceting natural diamonds is seen by comparison—and there can be little doubt that the technological problem of synthesizing large diamonds has been solved, although not yet as an economically viable process; it is still cheaper to dig them up.

Commercially valuable processes of this kind are normally protected by patents, and, predictably, the advent of synthetic diamond production in Sweden, South Africa, Japan and the Soviet Union brought headaches for the interested parties. It is in this context that the experiments described by Francis Bundy on page 116 of this issue of *Nature* will arouse concern. At what percentage, one may ask, does a contaminant become a constituent? The communication emphasizes the serious problems which still beset workers in the high pressure field, and the prime importance of painstaking experimentation. The pre-eminent position of General Electric in this field for the past twenty years must ensure that their results are taken seriously.

The fact that traces of nickel may be decisive for diamond synthesis is of interest also in the geological context, because although all can study pieces of the

Moon, the Mohole project has not yet delivered a stick of upper mantle rock. Some of the best preserved mineral grains available to geologists at the present time from high pressure environments are in fact the crystalline inclusions in diamond; the commonest of these inclusions is olivine, and six olivines (three from Venezuela and three from Ghana) microprobed at the Geophysical Laboratory in Washington showed not only an extremely constant ratio Fe/Mg (about 8 per cent Fe), but also an extremely constant Ni content, at about 5 per cent of the Fe content. As the 1959 paper put it, "diamond can form in several different ways, and stubborn mysteries still surround some of them". Diamonds aside, geologists as a whole owe much to General Electric. Maybe it is time that somebody named a new mineral for them.

—H. J. M.

Sequencing DNA

METHODS, notably those devised and developed during the past several years by Sanger's group at the MRC Laboratory of Molecular Biology in Cambridge, which allow the determination of the nucleotide sequences of RNA molecules have become part of the stock in trade of molecular biology laboratories. Announcements of the determination of the complete base sequence of this or that transfer RNA or other small RNA molecule have thus become almost commonplace and cause only a ripple of general interest, although messenger RNA molecules, whose sequences molecular biologists would dearly like to know, cannot usually be purified to the standards required for sequence analysis. In short, the methodology for sequencing RNA molecules is at hand and the chief obstacles to analysing many interesting RNA molecules lie in their purification.

By contrast, methods for analysing the base sequences of DNA molecules are still in their early infancy and most groups intent on determining the nucleotide sequence of short fragments of DNA have shirked taking the bull by the horns and have instead adopted indirect approaches. Usually these methods involve either transcribing *in vitro* with *Escherichia coli* RNA polymerase the DNA of interest and then analysing the base sequence of the RNA transcript, or using *E. coli* DNA polymerase I to synthesize a labelled complementary DNA chain which can be analysed. The obvious disadvantage of these sorts of experiments is that they rely on the fidelity of *in vitro* transcription or DNA synthesis, which is not the least of the reasons why Sanger and his colleagues have tackled the problem of devising a generally applicable methodology for the direct analysis of the base sequences of DNAs. To judge from what Ziff *et al.* and Robertson *et al.* had to say in last Wednesday's *Nature New Biology* (241, 34 and 38; 1973) they have succeeded in their objective.

The first problem facing anybody who tries to analyse the base sequence of a naturally occurring DNA molecule is that of devising methods for partially digesting the DNA into specific fragments small enough to analyse; the smallest DNAs, phage genomes, are at least 5,000 bases long. Ziff *et al.* used T4 phage endonuclease, which preferentially attacks single-stranded DNA, to digest par-

tially single-stranded ϕ X174 DNA in conditions which favour the formation of DNA secondary structure. They then separated by gel electrophoresis the fragments of ϕ X174 DNA, which because of the specificity of T4 endonuclease have a 5' cytidine residue bearing a 5' phosphate group and isolated two for further analysis. Robertson *et al.*, on the other hand, used a technique devised by Steitz for the sequence analysis of the ribosome binding sites of phage R17 RNA to obtain a fragment of ϕ X174 DNA. They bound 70S ribosomes to the single-stranded ϕ X174 DNA, using conditions Bretscher had devised to bind ribosomes to DNA, and then they digested away all the ϕ X174 DNA that was not protected by the bound ribosomes. The ribosomes were then dissociated and the protected fragments of DNA released were then subjected to gel electrophoresis and chromatography. The population of fragments of DNA proved to comprise one large portion about fifty nucleotides long and several smaller portions; the principal portion was isolated for further analysis. Clearly both these methods for obtaining small fragments of DNA have their limitations and no doubt in the future enzymes such as the bacterial restriction endonucleases, which can cut double-stranded DNA at specific sites, will be used by DNA sequencers.

Having obtained a fragment of ϕ X174 DNA of manageable size, Ziff *et al.* began the task of analysing its sequence. The procedure which they devised involves depurination and further digestion of the fragment with T4 endonuclease, snake venom phosphodiesterase and spleen phosphodiesterase coupled with standard fingerprinting chromatography. Using these procedures the group obtained the base sequences of a series of overlapping oligonucleotides and hence the sequence of the forty-eight nucleotides in the fragment of ϕ X174 DNA.

By exploiting these procedures and in addition using streptococcal nuclease Robertson *et al.* determined the sequence of the fifty-one bases of the strong ribosome binding site in ϕ X174 DNA which they had isolated. This sequence is interesting because it contains an ATG triplet which is, of course, the equivalent of an AUG codon in messenger RNA. Furthermore, if the base sequence is read in triplets, starting with the ATG triplet, an amino-acid sequence can be predicted. This predicted sequence is identical to that at the amino-terminal end of the ϕ X174 spike protein specified by cistron G which Air and Bridgen determined once Robertson *et al.* had begun the analysis of their fragment of ϕ X174 DNA. Together these results unambiguously prove that the fragment of the ϕ X174 genome which Robertson *et al.* have sequenced is the beginning of the G cistron.

This sequence, as Robertson *et al.* point out, has several striking features in common with the sequences of some of the ribosome binding sites of the RNA phages R17 and Q β . As they say, "Thus a pattern of similarities seems to be emerging among known ribosome binding site sequences which may in the future be explained in terms of ribosome or factor specificity and thus play an important part in the control of initiation of protein synthesis". But if realization of the significance of these initiation site sequences is something for the future, the methods that Ziff and Robertson and their colleagues have pioneered can be immediately exploited to yield information about the base sequences of many DNAs, for they have the twin advantages of being direct and being generally applicable.

—From a Correspondent.

Climate and Ecological Change in East Africa

IN 1962, Livingstone (*Nature*, **194**, 859) produced the first piece of direct evidence that 15,000 years ago the glaciers of Ruwenzori in East Africa were retreating at the same time as the Würm Glacier of Europe and the Wisconsin of America. Coetzee (*Nature*, **204**, 564; 1964) produced similar evidence for Mt Kenya, and Kendall (*Ecol. Monog.*, **39**, 121; 1969) has demonstrated climatic fluctuations in the Lake Victoria Basin. Large climatic changes in the comparatively recent past have clearly demonstrated that the vegetation and attendant faunal fluctuations must have been a marked phenomenon over most of the African continent. Indeed, it may well be that the Pleistocene overkill (Martin, *Nature*, **212**, 339; 1966) may be explained as much by climate as by human factors.

Climatic changes such as these may be considered to be recent in a geological sense, although in terms of present-day ecology they may be considered historic. If, however, the climate of the past hundred years in eastern Africa is examined, there is strong evidence to suggest that, superimposed on these longer term fluctuations, there are short-term changes that are of real significance to workers in the field of wildlife management today.

Western and Van Praet, on page 104 of this issue of *Nature*, convincingly demonstrate cyclical changes in both habitat and climate of the Masai Amboseli Game Reserve. The loss of fever trees (*Acacia xanthophloea*) from the basin has been noted over twenty years and especially during the past ten. The loss of this vegetation has been the cause of much concern, and Masai cattle and the elephant population have been blamed for the decline in tree density.

The whole Amboseli basin, which contained a large lake in the late Pleistocene, dried up during the Recent epoch. Western and Van Praet have found that the number of dead trees increases towards the basin centre and, because Masai cattle have been excluded since 1961, they conclude that domestic stock are not

related to this tree death. Eighty-five per cent of the trees in the basin show damage from elephants, so that it might be presumed that they could be considered as being the primary cause of tree death. Seventeen per cent of the dead and dying trees are undamaged by elephants, however, so that Western and Van Praet looked for another primary factor, and demonstrate that increasing salinization of the soil towards the basin coincides with high densities of dead trees. This increase in salination is associated with raised water levels and can be correlated in this closed drainage system to variations in rainfall.

Western and Van Praet show that Amboseli is sensitive to variations in water level which are indicated by alternation of hydrophytic and halophytic vegetation. Evidence from the oral history of Masai age sets clearly demonstrates that a halophytic vegetation covered the basin during the past century with a hydrophytic woodland forming in the first quarter of the present century and a strong reversal of this trend during the past ten years. These changes may well be shown to be the result of a long-term fluctuation in rainfall with a period of about 100 years. Thus in the closed drainage system of the Amboseli basin, and perhaps in similar areas such as Lake Manyara in East Africa, the primary cause of vegetation change seems to be rainfall, with elephants merely acting in a catalytic manner to accelerate changes already taking place.

Laws (*Oikos*, **21**, 1; 1970) has demonstrated that elephants act as important agents of change in many East African habitats especially in the conversion of woodland to grassland as has been observed in the Murchison National Park in Uganda and the Tsavo National Park in Kenya. In Tsavo these changes have been dramatic and a recent drought has led to the death of up to 5,000 animals. It has thus been suggested that the high elephant density and encroachment by man have been the primary causes for these changes.

Although it is quite clear that the influence of man on the distribution of large mammals has been profound in many places, Western and Van Praet's article demonstrates the caution that must be used when interpreting vegetation changes in terms of man and animal numbers alone, for although to a large degree their influence is everywhere profound, the action of long- and short-term climatic changes may yet be shown to be a primary factor in the dynamics of these areas of variable and extreme climate.

The emergence of independent East African states with their rapidly expanding populations makes it imperative that the best possible economic use be made of the land. Vast tracts of these nations, however, fall into a semi-arid classification, so that such economic exploitation must of necessity be combined with a high degree of ecological planning and common sense. In recent years, the income that Kenya derives from tourism now heads their list of foreign earnings. This important fact clearly demonstrates that the sound management of game reserves, national parks and other amenity areas may well form the basis of future development and a sound economy.—M. C.

PROTEINS

In vitro, or Almost

from our Cell Biology Correspondent

THE clawed toad, *Xenopus laevis*, has, of late, come to occupy a new ecological niche—the laboratories of molecular biologists many of whom, I suspect, have not since their boyhood days of jam jars full of frogs' spawn and tadpoles given amphibians a second thought. The reason for this sudden interest, from such an unexpected quarter, in *Xenopus* is that Gurdon and his school and collaborators have shown recently that globin messenger RNAs and calf lens crystalline messenger are, when injected into *Xenopus* oocytes, translated with fidelity and at a reasonable efficiency. In other words, *Xenopus* oocytes provide what almost amounts to a cellular cell-free system for studying the translation of at least

some eukaryotic messengers, and in many laboratories any available mRNAs are being injected into these cells in the hope that they will be translated. It is hard to say what the failure rate is, but as Laskey, Gurdon and Crawford report in the current issue of the *Proceedings of the National Academy of Sciences* (69, 3665; 1972), the single-stranded RNA genome of encephalomyocarditis (EMC) virus, a picornavirus related to polio virus, can definitely be added to the list of successes.

After EMC RNA is injected into oocytes in the presence of 35S methionine, polypeptides not present in oocytes injected with saline are made. As polyacrylamide gel electrophoretic analysis reveals these newly made polypeptides have identical mobilities to the EMC proteins made when the virus replicates in ascites cells. The fact that tryptic fingerprints of three of the polypeptides made in oocytes are identical to the fingerprints of the co-electrophoresing EMC proteins made in ascites cells leaves no room to doubt that in oocytes EMC RNA is not only extensively but also faithfully translated.

That EMC RNA is translated in *Xenopus* oocytes is not particularly surprising, but it is interesting that in these cells, as in ascites cells, which support virus replication, the polypeptide product of translation is processed after translation. There is good evidence that EMC RNA, like polio virus RNA, is translated, during virus replication in mammalian cells, into a single large precursor polypeptide that is subsequently cleaved to yield the mature viral proteins. These mature proteins can be detected in extracts of oocytes injected with EMC RNA so that it must be assumed that they arise by specific cleavage of the primary product of translation. This obviously implies that oocytes contain proteases with specificities similar to those in mammalian cells and that mechanisms for the post-translational cleavage of polypeptides may be widespread among eukaryotic cells. Whether EMC can be persuaded to replicate in oocytes to yield infectious progeny virions remains to be seen.

Laskey *et al.* point out the advantages of the oocyte system over cell-free systems from ascites cells but the latter have their adherents and uses. Oberg and Shatkin (*ibid.*, 3589), for example, have used an ascites cell-free system programmed with EMC RNA to obtain further evidence for a unique initiation site for translation of this messenger. They fingerprinted the polypeptides made in the presence of 35S Met-tRNA_{Met} and 35S Met-tRNA_{P^{Met}}; the latter tRNA translates only initiating AUG codons and fingerprints of polypeptides made in its presence reveal a

single N terminal tryptic peptide containing 35S methionine. In other words, in this cell-free system, translation of EMC RNA and the RNAs of mouse Elberfeld virus and mengo virus is initiated at a unique site.

It remains true, of course, that compared with *Escherichia coli* cell-free systems for *in vitro* protein synthesis both ascites cell systems and the oocyte injection system are in their infancy, and one of the most remarkable results obtained of late with the *E. coli* system is that reported last April by Siegert *et al.* (*ibid.*, 888). They found that in such a system the RNA of avian myeloblastosis virus is translated faithfully to yield four of the group specific antigens of the avian RNA tumour viruses. Gielkens, Salden and Bloemendal (*FEBS Lett.*, 28, 348; 1972) now report that RNA from Rauscher leukaemia virus and mouse mammary tumour virus stimulates polypeptide synthesis in an *E. coli* cell-free system. Furthermore, at least some of the smaller polypeptides which are made co-electrophorese with proteins of the respective virions, and polypeptides specified by the leukaemia virus are not related to those specified by the mammary tumour virus. By contrast, globin messenger and calf lens crystallin messenger stimulate very little protein synthesis and the short peptides which are made are the products of aberrant initiation.

Gielkens *et al.* also mention that in collaboration with Gurdon they have injected Rauscher leukaemia virus RNA into oocytes, but they failed to detect evidence of its translation. It seems therefore that for RNA tumour virologists the *E. coli* cell-free systems hold great and unexpected interest.

Aminoacylation of Viral RNAs

ONE of the most intriguing features of the single-stranded RNA genomes of plant viruses such as turnip yellow mosaic virus (TYMV) and tobacco mosaic virus (TMV) is the occurrence at the 3' end of these RNAs of the sequence . . . pCpCpA. This same 3' terminal sequence is common to all transfer RNAs and, like transfer RNA molecules, the RNAs of TYMV and TMV can be esterified by an amino-acid. TYMV can be specifically charged with valine and TMV can, as Litvak *et al.* report in next Wednesday's *Nature New Biology* (January 17), be specifically charged with histidine using yeast histidyl-tRNA synthetase to catalyse the reaction. Furthermore, Litvak *et al.* have found that both histidyl TMV RNA and valyl TYMV RNA can under appropriate conditions react with the protein synthesis elongation factor 1 (EF1) purified from wheat and with

IMMUNOLOGY

Antigens and Ageing

from our Cell Physiology Correspondent

It is always interesting when results emanating from different laboratories are diametrically opposed to one another, but when such conflicting reports are published in the same journal the reader finds it hard to know what to believe. In a recent issue of *Experimental Cell Research* two groups report on their studies of the fate of HLA antigens in ageing diploid cells; one group comes to the conclusion that these change with age whereas the other group believes that there is no change.

The HLA antigens have long been recognized as being useful for distinguishing between the tissues of genetically different individuals and it is also known that diploid fibroblasts retain the HLA antigens of the individual from whom they were derived. This fact is often taken to be an indication of the normality or non-neoplastic nature of the cells, just as the fact that the cells can only be subcultured a limited number of times, after which degenerative changes are evident, is thought to be akin to what is understood as ageing.

Sasportes and his colleagues (*Nature*, 233, 332; 1971) found that in some ten strains of fibroblasts obtained from skin biopsies, HLA antigens were lost as the cells grew older, the loss of antigens being evident before observable degenerative changes in the culture. Before this report appeared it had generally been accepted that histocompatibility antigens were retained throughout the life span of the cells, there being no change in the expression of HLA even during prolonged cultivation. Work

GTP to form a ternary complex of EF1.GTP.aminoacyl viral RNA. But TYMV RNA and TMV RNA that is not charged with an amino-acid does not react with EF1 and GTP.

Apparently the 3' terminal sequence of these viral RNA genomes allows these RNA molecules to be acylated with an amino-acid and subsequently to form a ternary complex with an elongation factor. These events, as Litvak *et al.* speculate, may play a crucial part in regulating either the initiation of replication of these RNAs or the initiation of their translation by the protein synthesizing machinery of host plant cells; for example, the complex of viral RNA with elongation factor may have a high affinity for ribosomes or, by analogy with the replication of phage Q β RNA, elongation factor 1 may form part of a multi-meric viral RNA replicase.

recently carried out in Hayflick's laboratory by Brautbar and his colleagues (*Exp. Cell Res.*, **75**, 31; 1972) supports the idea that there is no loss of HLA antigens in ageing diploid cells, for with cultures of WI38, WI26 and MRC-5 the same characteristic "tissue-type" was found even during the final senescent stages.

By contrast, Goldstein and Singal (*ibid.*, 278) report that in the strains of diploid fibroblasts which they have examined, loss of HLA antigens does occur and, as Sasportes found, this loss is detectable before the degeneration of a culture. In fact, it can even be used to diagnose the impending senescence of a culture.

The discrepancy between these various reports may have some purely technical explanation, the most likely one being that Brautbar and his colleagues examined only mass cultures of cells (Goldstein set out to look at cell clones rather than mass cultures). Indeed, because the mass cultures from which clones were derived showed no loss of HLA antigens it can be suggested that changes of antigen may be obscured when cells are grown *en masse*—probably as a result of the cooperation which is known to occur between cells in crowded conditions.

The method of assessing HLA may also have something to do with the discrepancy, for although Sasportes and Goldstein both used a dye exclusion method, Brautbar adopted a fluorochromatic one. Moreover, it is possible that the method of trypsinization may affect the result, for it is well known that the enzyme removes antigens from the surface of cells and it is therefore possible that the time at which HLA antigens are assessed after treatment is critical.

But can one interpret three negative reports and two positive ones in favour of the positive? The evidence which Goldstein provides with cell clones strongly suggests that one can, and it is interesting that with the different clones derived from a single cell strain the same HLA antigen is not always lost. Possibly this will support ideas which suppose that the molecular basis of ageing is of a random nature.

More important, however, is the fact that both Sasportes and Goldstein find that the loss of antigens is predominantly from the second of the HLA loci, the locus which is generally considered to be of greatest importance in so far as cell recognition is concerned. Might these results not therefore turn out to be a profoundly important link in what is known about ageing? If the loss of HLA antigens on these diploid fibroblasts reflects loss of antigens and surface membrane changes *in vivo* then it has important implications. Changes affecting either lymphoid or non-

lymphoid cells would result in problems of self-recognition and loss of immunosurveillance so that autoimmune or neoplastic disease might follow.

True, it has yet to be established beyond any doubt that loss of HLA antigens or surface membrane changes do occur, but it is most attractive to interpret Sasportes's results and these recent results of Goldstein's in terms of immunological theories of ageing.

MEMBRANES

Lipophilia

from our Molecular Biology Correspondent

CURRENT beliefs about the state of proteins in membranes are encapsulated in artistic representations chiefly to be found in the pages of conference reports, in which they appear rather like marshmallows floating in a sea of treacle. The evidence is that, depending on the composition of the membrane, they can drift around with greater or lesser freedom, but that their orientation relative to the bilayer plane remains essentially invariant. There is, in other words, a large energy barrier to inhibit tumbling motion. There is also the possibility that the protein molecules attract around themselves a mantle of one or more of the phospholipid components of the bilayer, with which they associate relatively strongly. Practically nothing is known about the degree of specificity of protein-lipid interactions in membranes, or yet of their character and mechanism. There is scope for reasonable conjecture, not to say notions on the wilder fringes,

involving such extravagant concepts as inside-out globular proteins.

One of the more conservative experimental approaches is to find a membrane enzyme which can be reversibly stripped of its lipids, and then use its activity after recombination with selected lipid components to assess the degree of specificity of its association with the bilayer constituents. In general the upshot has been that the requirements are only moderately specific. A very clear-cut and instructive new example of such a system comes from Garland and Cori (*Biochemistry*, **11**, 4712; 1972), working with the microsomal enzyme, glucose-6-phosphatase. The active preparations are lipoprotein particles, which lose their activity on treatment with phospholipases. These enzymes, however, are not nice to use in such a situation, on account of requirements for calcium ions or of the release of hydrolytic products, both of which complicate the interpretation in a disagreeable manner. What Garland and Cori have found is that under carefully defined conditions, non-ionic detergents, and in particular deoxycholate at sufficient concentration, permit reversible separation of the lipid. When the resulting mixture is applied to a 'Sephacrose' column, the matrix of which excludes only very large particles, some of the protein, together with all the enzymatic activity, elutes in the void volume, but the bulk of the protein and nearly all the phospholipid are retarded, and emerge with different elution profiles. This protein fraction, almost free of lipid and inactive, can be completely reactivated by addition of new lipid. This only works, however, if some deoxycholate is kept in the mixture, or to

Structural Probes and Peptides

IN next Wednesday's *Nature New Biology* (January 17) Beyer, Craig and Gibbons describe an extension of the application of fluorescent probes of protein structure to oligopeptides. The dyes, anilino-naphthalene sulphonic acid and *p*-toluidinylnaphthalene sulphonic acid, are widely used as markers for local conformational changes in proteins. Their fluorescence is greatly enhanced when they bind on low-polarity sites, such as active-centre cavities, in the protein molecule. Using thin-film dialysis and fluorescence spectroscopy Beyer *et al.* studied the interaction of one of the dyes (TNS) with the cyclic antibiotic peptides, tyrocidines A, B, and C, gramicidin S-A, and bacitracin A. In thin-film dialysis the escape rate of a given molecule through a membrane is proportional to the concentration of that molecule on the inside.

With a membrane that passes free

TNS much faster than the oligopeptides, Beyer *et al.* found that the escape of TNS is noticeably retarded by all the above peptides, as well as linear peptides, but not by succinyltyrocidine B. This indicates that coulombic interactions play a significant part in the binding process, for the effect of succinylation is to convert a positive charge, on an ornithine side chain, which would interact favourably with the sulphonyl group of the dye, into a negative charge.

In spite of the fact that there is interaction with the whole range of peptides, fluorescent enhancement was observed only in the presence of the tyrocidines. These are distinguished from the others by their tendency to self-association in aqueous solution. The associated form evidently provides an environment in which the TNS may be sequestered, so as to generate large quantum yields.

a lesser extent, if the phospholipids are first sonicated.

If, on the other hand, the bile salt concentration is too high, the activity falls catastrophically. Different phospholipids varied considerably in their ability to engender reactivation. The mono-unsaturated phosphatidylcholine is most effective, and generates full activity at half the concentration of total lipid present in the original preparation. The saturated version, dipalmitoyl-phosphatidylcholine, by contrast induces no activity whatever. The doubly unsaturated dioleoyl derivative behaves similarly to the mono-unsaturated species. There is some precedent for this type of specificity relationship in regard to other membrane enzymes, but the mechanism is obscure. The role of the bile salt in promoting reactivation must be supposed to reside in its ability to destabilize the phospholipid micelles, and so make monomers available for reaction with the protein.

A different path towards the study of lipid-protein interactions has been pursued by Marchesi and his colleagues, who have been studying the red cell membrane glycoprotein (so-called glycophorin), which carries cell surface receptor groups. This molecule probably has a molecular weight of about 50,000, contains 60 per cent by weight of carbohydrate, and, so the story goes, is the most integral of membrane proteins, in that it projects right through the bilayer, whereas the bulk of red cell membrane proteins are to be found at the inner surface.

Segrest *et al.* (*Biochem. Biophys. Res. Commun.*, **49**, 964; 1972) have now sequenced a large tract of this molecule, which is evidently the very part that is normally lodged in the lipid interior of the bilayer. They have obtained cyanogen bromide and tryptic peptides with a good overlap. Two fragments are sialoglycopeptides, and therefore from the N-terminal end of the protein, which lies on the outer cell surface. Another is the C-terminal tract, which overlaps with a long tryptic peptide. Between them they define a sequence of fifty-one residues, all but the C-terminal end of which must be presumed normally to reside within the membrane bilayer. Twenty-three successive residues in the sequence are hydrophobic. At either end there is a considerable cluster of charged side chains, and it is reasonable to infer that these may be the parts of the protein that project into the aqueous media on the inside and outside of the cell. Segrest *et al.* refer to evidence of their own, as yet undivulged, that the hydrophobic stretch is an α -helix. Its length in this case would be some 35 Å, which is about right to span the bilayer. If the authors can confirm this model, it will clearly be a result of great interest.

LASSA VIRUS

A New Disease of Man?

from our Medical Virology Correspondent

THE name "arenavirus" defines a group of RNA viruses with a mean diameter of 110–130 nm, a dense well-defined envelope covered with projections and containing a variable number of electron-dense granules, giving a sandy appearance from which the generic name is derived (*Arenosus*, L. sandy). Other characteristics separate the arenaviruses from RNA lipid solvent-sensitive viruses. The group includes lymphocytic choriomeningitis virus, the Tacaribe complex viruses (haemorrhagic fever viruses of South America) and the recently described Lassa virus, all of which cross-react antigenically.

The dramatic story of Lassa fever began when two missionary nurses

from Lassa, in north-east Nigeria, died in 1969 from a mysterious illness, and a third nurse, who was gravely ill, was flown to the United States. This nurse recovered and convalescent plasma from her was effective in the treatment of a laboratory worker, who acquired the infection while working with tissue cultures infected with blood from these patients (J. D. Frame *et al.*, *Amer. J. Trop. Med. Hyg.*, **19**, 670; 1970). The virus was isolated and characterized (S. M. Buckley and J. Casals, *ibid.*, 680) and Lassa fever was established as a new virus disease of man.

A further outbreak of Lassa fever, with a high mortality of 52 per cent among twenty-three patients admitted to hospital, was reported in Jos, Nigeria, in 1970 (H. A. White, *Trans. Roy. Soc. Trop. Med. Hyg.*, **66**, 390; 1972). Dr Jeannette M. Troup, who performed two autopsies while investigating Lassa

Third Type of Pyroclastic Rock

PYROCLASTIC rocks—fragmental volcanic products ejected from volcanoes in explosive events—have usually been divided into two categories, fall deposits and flows. But in next Monday's *Nature Physical Science* (January 15) Sparks and Walker propose a third category, that of ground surge deposits. This category includes some deposits at present classified as pyroclastic flows—frothy, gas-filled glassy lava which bubbles from a volcanic vent during eruptions—and some deposits of *nuées ardentes*, which include the glowing avalanches that dominate the popular picture of volcanic eruptions.

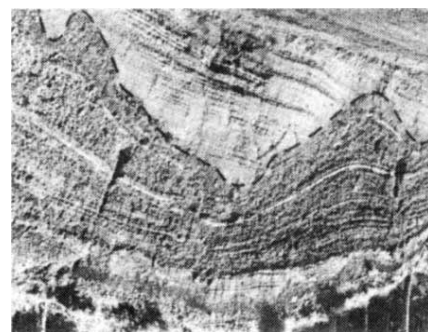
The ground surge deposits are thus characterized by flows along the surface of the Earth, and are clearly distinguished from fall deposits, which show the effects of their passage through the air after ejection, for example in their internal stratification (see figure). But why is it necessary to distinguish between the new category and the pyroclastic flow category?

Active pyroclastic flows are believed to be fluidized by dissolved magmatic gas or by air trapped beneath the advancing flow. They travel as a dense mass, and pumice floats to the top of the flow, where it is preserved when the lava cools to form solid rock. These characteristics result in a structure which mantles the topography around the volcanic vent with a roughly uniform layer of rock.

Although ground surge deposits also mantle the topography, their thickness is not uniform—"they show a pinch and swell structure", as Sparks and Walker put it. Furthermore, they have an internal stratification which is not parallel to the bottom and top of the layer, and

they extend out to only a few kilometres from the source. They are relatively thin—less than 1 m compared with the thickness of 10 m or more commonly associated with pyroclastic flows—and they can occur on relatively steep slopes ($>10^\circ$) where pyroclastic flows do not solidify.

The overall picture painted by Sparks and Walker is one in which explosive volcanic activity produces, in addition to the ejecta thrown high into the air, both a dense flow, which chiefly follows the valleys, and a relatively low density ground surge, or "ash hurricane", akin to the ground surge generated in a nuclear explosion. The resulting thin ground surge deposit is very susceptible to erosion, and survives only when covered by other volcanic deposits; that explains why it has taken so long for the ground surge to be recognized as a separate category. Finally, the pinch and swell structure can be accounted for by a wave pattern, with wavelengths found so far in the region 4 m to 45 m.



Pyroclastic fall deposits. Dashed line is an erosional unconformity; the fall deposit above it shows internal stratification common among such deposits.

fever and who was primarily responsible for drawing attention to the condition, contracted the infection and died of it.

In March 1972 further cases of Lassa fever occurred among four patients and seven members of the staff in a hospital in the Zorzor district of Liberia in West Africa, and four died. The index case was a pregnant woman admitted to the obstetric ward, and all cases among the patients and members of the staff were in this ward. One of the fatal cases was an American missionary nurse who had direct contact with the blood of the index case (*Morbidity and Mortality Weekly Report*, **21**, 237; 1972). A more recent report (*ibid.*, 386) records a further sixty-four cases of Lassa fever admitted to hospital in Sierra Leone. Twenty-three (36 per cent) died, and the case fatality ratio among pregnant women was 75 per cent (six out of eight). One of the sixty-four cases was a nurse who pricked her finger on a needle used for obtaining blood from a patient who subsequently died of clinical Lassa fever.

This is the largest yet reported epidemic, and, unlike the previous outbreaks in Nigeria and Liberia, it consisted primarily of community-acquired infection. Evidence was obtained of family outbreaks of the fever in which spread has occurred among those with the most intimate contact. Man-to-man spread of Lassa virus undoubtedly takes place through contact with blood or infectious secretions, but a reservoir of infection has yet to be identified. Lassa virus is antigenically related to the rodent-associated haemorrhagic viruses of South America and to lymphocytic choriomeningitis virus, usually an inapparent infection of wild mice, but an animal source of Lassa virus or antibodies to this virus in wild rodents has not been found.

It is interesting that many features of this infection are reminiscent of the green monkey disease or Marburg virus infection (see *Nature*, **233**, 236; 1971), although, fortunately, no further cases of Marburg disease have been recorded since 1967. In the meantime, however, Lassa fever looms as an awesome new viral infection in tropical Africa, and the epidemiological propensities of this infection may be very wide with the modern means of fast air travel.

MINERAL RESOURCES

Boron from the Sea

from our Soviet Correspondent

NEW work on the sorption of boron compounds may provide a way of isolating the element from seawater. Although the concentration of boron in

seawater is relatively low (8 to 9 g B_2O_3 m^{-3} in the Black Sea and 15 to 16 g B_2O_3 m^{-3} in the oceans), the estimated total content of boron in the seas and oceans (2.2×10^{13} tonnes B_2O_3) has provoked a considerable amount of research on the subject. New findings by Nikolaev of the Institute of Inorganic Chemistry of the Siberian Branch of the Soviet Academy of Sciences and Ryabinin of the Hydrophysical Marine Institute of the Ukrainian Academy of Sciences suggest a means of recovering boron, using ZrO_2 as a sorbent (*Dokl. Akad. Nauk SSSR*, **207**, 149; 1972).

Nikolaev and Ryabinin carried out a detailed survey of salinity, pH and temperature conditions affecting the sorption of boric oxide on various hydrated oxides, for different initial concentrations of the boron. Best results were obtained using hydrated zirconium oxide as sorbent and a pH value of 8 to 9—close to that of natural seawater. The effectiveness of the process was found to increase continuously with the amount of sorbent, practically total extraction of the boron (98 per cent) being obtained for a ratio of ZrO_2 to B_2O_3 of 400.

The experiments were performed with water from the Black Sea, and the good results obtained, even at these low concentrations, lead Nikolaev and Ryabinin to think that the use of a zirconium oxide sorbent may be an economically realistic method of recovering boron from the sea.

SEMICONDUCTORS

Transistor Revitalized

from a Correspondent

It seems that the transistor, which only a few years ago was considered by many people to have reached the limit of its performance, is now a serious contender in the microwave region and may well extend its range to about 10 GHz. This was the theme of a timely symposium held at Imperial College, London, on December 13, and arranged by the Electronics Group of the Institute of Physics in collaboration with the Institute of Electrical Engineers.

Many of the contributions reported significant advances in both silicon and gallium arsenide technology. In particular two speakers, D. J. Hinds (GEC Hirst Research Centre, Wembley) and Dr R. P. Arrowsmith (Post Office Research Department, Dollis Hill, London), discussed recent advances in the technology of silicon microwave bipolar transistors. It now seems that arsenic has replaced the more conventional phosphorus as the emitter dopant in npn devices. Improved cut-off frequency (f_T) and noise performance result in useful performance up to 4 GHz with promise of extending this to at least 8 GHz by reverting to an improved version of the old "mesa" design.

Many of the improvements can be attributed to great advances in optical photolithography, which mean that line

Laboratory-made Chert

THE flint-like rock called chert which has turned up in surprising quantities beneath the Atlantic to blunt the drilling bits of the Glomar Challenger research vessel continues to hold the attention of geologists. Last year Weaver and Wise of Florida State University reported that some of this chert, which is also found in other oceans, is composed of blades of cristobalite 300 Å to 500 Å thick gathered together in balls about 10 μm across (*Nature Physical Science*, **237**, 56; 1972).

These fascinating structures have now been reproduced in the laboratory. In an article in next Monday's *Nature Physical Science* (January 15), J. H. Oehler (University of California at Los Angeles) reports experiments in which aliquots of silica dispersed in water were subjected to temperatures of 150°C and pressures of 2 kbar for four weeks. The product, examined under the scanning electron microscope, consisted of 50–75 μm balls of platy, apparently hexagonal, crystals (see figure).

Oehler says that this hexagonal appearance of the artificial chert is



characteristic of tridymite rather than cristobalite, which forms octahedral crystals, and he favours the view that what is being formed are hybrid crystals composed of layers of both tridymite and cristobalite, the tridymite structure dominating the morphology. X-ray diffraction data have so far been unable to confirm or deny the hypothesis. Oehler believes that this layered structure may well apply to the cristobalitic cherts recovered from the oceans.

widths for the emitter stripes in interdigitated structures of the order of $1\text{ }\mu\text{m}$ are now possible. To extend performance even further would require the use of electron beams to generate the mask patterns and possibly soft X-rays to project these patterns onto the semiconductor slice. New technologies such as ion implantation and proton-enhanced diffusion are being developed so that the doping profiles can be accurately controlled, for base widths as small as $0.1\text{ }\mu\text{m}$ are required.

R. C. Aubusson (Ferranti Ltd, Manchester) considered the use of polycrystalline silicon as an overlay for the emitter contact sites in order to introduce current sharing resistors to reduce the formation of hot spots and thus diminish the risk of thermal overload damage. Using this technique some 5 W at 2 GHz is now available. T. C. Denton (GEC Hirst Research Centre, Wembley) discussed the introduction of similar current sharing resistors into a mesh emitter silicon transistor, which allows pulse powers of the order of 150 W at 1 GHz to be obtained. Further advances in the gallium arsenide field effect transistor (FET) were described by J. A. Turner (Plessey, Allen Clark Research Centre, Caswell); changes in the structure from a one layer to a two layer epitaxial device have improved the mobility of the carriers in the active layer so that useful gain and noise performance up to 10 GHz is now available. Again the results are dependent on narrow gate widths of the order of $1\text{ }\mu\text{m}$ and further improvements depend on the smaller dimensions available from masks generated by electron beams.

Significant also was the extent to which computer simulation and new theories about the internal physics of the devices have been able to explain discrepancies between previous predictions and the actual performance of devices as reported by J. A. G. Slatter (Mullard Research Laboratories, Redhill). There has long been an unexplained difference between the predicted cut-off frequency (f_T) and current gain for bipolar silicon microwave devices, and a significant contribution from Dr H. De Man and R. Mertens (Laboratorium Voor Fysika En Elektronika Der Halfgeleiders, Leuven) showed that the phenomena can be explained by band tailing and formation of impurity bands. The effect produces a strong increase in the intrinsic carrier density in the emitter region and increases the charge storage of minority carriers, particularly in the emitter side wall of double diffused devices.

A further example of the use of computer simulation was given by J. C. Henderson, R. J. D. Scarbrough and

Dr J. Earney (Post Office Research Department, Dollis Hill) who compared two identical bipolar transistor models, one in silicon and the other in gallium arsenide. Here was a case where predictions and comparisons could be based on the most recent advances in the two technologies. A plea was made that the recent gallium arsenide laser technology should be extended to the field of bipolar devices where even better performance is predicted.

Dr M. Merkel (Nuclear Research Centre, Grenoble) discussed the problems that arise when the more normal one-dimensional computer models are extended to cover the two-dimensional properties of practical devices. Many of the characteristics of FETs, for example, can only be adequately described in this way and can pose difficult problems if economical computer programs are to be devised.

One of the remaining problems that confront the designer of devices is that of the reduction of the package limitations that restrict the inherent frequency and power output of the active device. This was the theme of a contribution by J. R. Twistleton (GEC Hirst Research Centre, Wembley) who pointed out that the effect of parasitic reactances is particularly severe in microwave power devices because of their inherent low impedance.

Surveyor Spacecraft Results Verified

ESTIMATES of the sizes of particles of lunar soil based on data relayed back to Earth by the Surveyor spacecraft, particularly Surveyor 3, have proved to be remarkably accurate. In next Monday's *Nature Physical Science* (January 15) Jaffe and Strand compare these calculations, made before lunar material was ever brought back from the Moon, with laboratory measurements of samples returned from the site of Surveyor 3 by the Apollo 12 astronauts (see diagram).

Methods used for the remote measurement of particle sizes included a measurement of the change in the photometric function of the surface when the soil was compressed by the spacecraft on landing. This led to the conclusion that the fine structure of the lunar material was smaller than 1 mm (which could just be resolved by the television cameras aboard Surveyor 3) and "larger than the order of $10\text{ }\mu\text{m}$ ". Yet other estimates involved the laboratory simulation of the reproduction in the lunar soil of tiny ridges 50 to $70\text{ }\mu\text{m}$ high on the bottom of one of the footpads of Surveyor 3.

When the relevant data from all Surveyor spacecraft are taken into account, the agreement with laboratory measure-

EARTH STRAIN

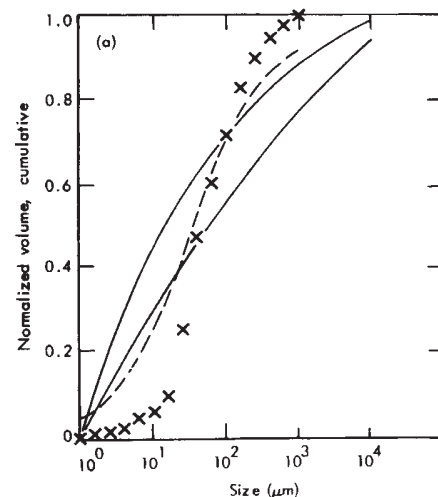
Inland Tides

from our Geomagnetism Correspondent

THAT part of the Earth between Bradford and Halifax in the north of England is unlikely to be subject to accumulation of strain in the long term, but, as Bilham *et al.* (*Geophys. J.*, **29**, 473; 1972) have now discovered, the region is not free from short term non-local variations in strain. So much, at least, is clear from a survey involving 28 months of continuous strain recording. In July 1969, Bilham and his colleagues installed a prototype invar wire strainmeter in the disused Queensbury railway tunnel which lies in the north-east—south-west direction. As it soon became clear, the instrument possessed several deficiencies which prompted the development of an improved version. In the meantime, however, the prototype was kept in operation and proved to have a sufficiently high signal-to-noise ratio to record Earth tides clearly and thus to demonstrate the importance of tidal loading at a site well inland.

The strainmeter itself, fixed to the tunnel wall by expanding rock bolts, comprised a 10 m invar wire maintained under constant tension by gravity acting on a steel weight. Strain changes in the Earth produced a small rotation of the tension weight, representing

ments is quite close in the middle of the logarithmic range of particle sizes, but diverges somewhat at each end of the range. Jaffe and Strand conclude that their results augur well for the determination of soil sizes on other planets by means of automatic spacecraft landing there.



X, Laboratory measurements reported by Jaffe and Strand; —, on-surface measurements (Surveyor 3); ---, on-surface measurements (all Surveyors).

extension or contraction depending on the direction. Because of the orientation of the tunnel wall an extension actually represented a load bearing in the north-to-east or south-to-west directions, whereas a contraction represented loads in the north-west or south-east quadrants. A transducer was used to convert the rotation of the weight into a variable voltage suitable for chart recording. The problem of friction in the transducer and in the coupling of the transducer to the weight was one of the shortcomings of the simple instrument. A more serious problem was posed by the friction of the pivot linking the weight to the invar wire, although the effects of temperature and pressure in the wire proved less serious, because they turned out to be small by comparison with the effects of tides except at low frequencies.

In spite of the problems, the recorded strain variations were of sufficient quality to warrant digitizing and spectral analysis. The chief interest of Bilham *et al.* was in the amplitudes of the various Earth tides and the degree to which the different components could be resolved, although they were also interested in studying the phase relationships between Earth tides as observed and calculated. To the latter end, therefore, it was necessary to determine Fourier components of the theoretical strain tide produced by the known motions of the Sun and Moon in a spherically symmetrical Earth having a distribution of elastic constants and density the same as that of the real Earth. This theoretical strain was calculated using the instantaneous tidal potential given by the positions of the Sun and Moon and the Love numbers calculated previously by Longman (*Geophys. J.*, **11**, 133; 1966). This allowed Bilham and his colleagues to generate a theoretical strain series over the same period as that over which a real strain was observed. Power spectra for both the observed and theoretical strain series were then determined, and all spectra were smoothed using a five-term Gaussian filter applied to the amplitude spectra.

The results showed that the smallest tides resolved (J and OO in G. H. Darwin's notation—*Scientific Papers*, **1**, 1; 1907) had strain amplitudes of 5×10^{-10} and that the frequency resolution was considerably better than Bilham and his colleagues had expected. The comparison between the observed and theoretical tides revealed the most striking difference in the properties of the diurnal and semidiurnal tides. The observed diurnal tides were roughly in phase with the theoretical tides, but had an amplitude lower by a factor of about three. The observed semidiurnal tides, on the other hand, agreed with the theoretical tides in amplitude but

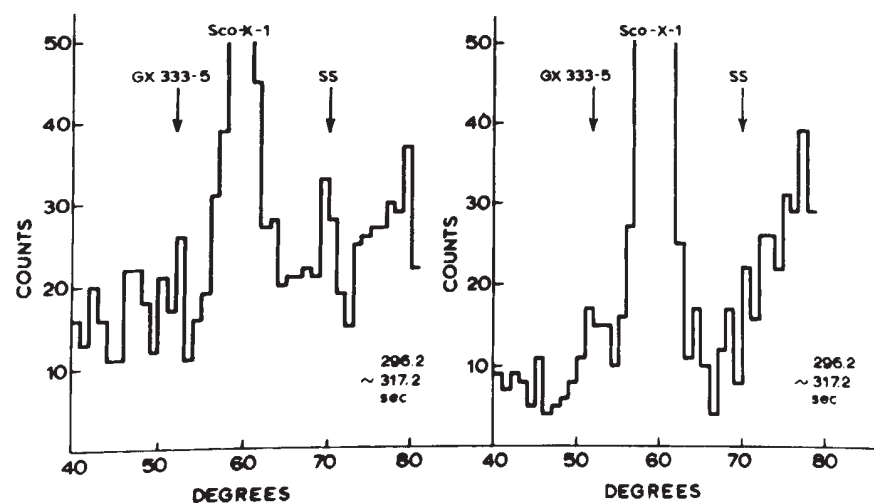
were about 180° out of phase. Such a difference in phase and amplitude could arise if the regional strain field were to be distorted by local inhomogeneities, although King (*Bull. Roy. Soc. New Zealand*, **9**, 239; 1971) concluded, on the basis of geological and rock mechanical evidence, that this should not happen at a site like Queensbury. Differences between observed and theoretical strains might also be observed if the fluid or solid Earth had some natural period of the order of 24 h or 12 h—but there are no such periods. Bilham and his colleagues are thus forced to the conclusion that the strain tides at Queensbury are strongly influenced by oceanic tidal loading.

This general conclusion is further supported by more detailed analysis. In simple terms, if the observed, theoretical and ocean load-induced strain signals are represented by functions of frequency— H , G and L , respectively—then L may be regarded as the difference between H and G , both of which are known. It is then possible to calculate the ratio of L and G ($=R$) which is clearly zero if there is no oceanic tidal loading at all. In fact, for semidiurnal tides, R ranges from 1.47 to 1.91 for the tidal peaks N , M_2 , S_2 and K_2 (Darwin

notation), thus clearly showing the dominance of the oceanic loading strains (L) over those produced by direct gravitational effects (G). For diurnal tides, R lies between 0.41 and 0.43, showing the oceanic loading effects to be smaller but far from zero. Some years ago, however, Cartwright (*Phil. Trans. Roy. Soc.*, **A263**, 1; 1968) analysed sea surface spectra for six tidal stations around the British Isles and also found that R is usually less than unity for diurnal tides.

The next step will clearly be to see if the implied oceanic tidal loading effects at Queensbury agree in detail with those predicted from real oceanic tides. So far this has not been done, but it is likely to be complicated because Queensbury will presumably be affected by tidal loading in both the North Sea and the Irish Sea and, to a lesser extent, in the North Atlantic. It is already clear, however, that strainmeters placed away from the coast can be used to investigate the regional behaviour of tides. And, as Bilham *et al.* point out, this could be of some social benefit, for inland strain stations could perhaps be used to detect tidal surges in the North Sea before they cause flooding.

Soft X-ray Source towards Galactic Centre



A ROCKET experiment flown on May 26, 1971, has led to the discovery of a discrete source of soft X-ray emission in a direction close to that of the galactic centre. In next Monday's *Nature Physical Science* (January 15) Bleeker and colleagues present spectra from several scans of the source and a best position error box. The source, designated SS, lies close to λ Sco, a bright spectroscopic binary of spectral type B1 V; if SS is at the distance suggested

for λ Sco by observations from OAO-2, then its luminosity in the band 0.37 to 1.9 keV is 2×10^{33} erg s $^{-1}$.

It is interesting that SS does not appear with any comparable brightness at hard X-ray frequencies. In the figure, the left-hand histogram is for a scan at 0.37 to 1.9 keV, and SS can be picked out readily; on the right, the same scan at 1.9 to 6.5 keV, SS is completely absent, indicating that the source has a very soft spectrum.

Leukaemia Antigens and Immunity in Man

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Dr Harris describes recent research into the causes of leukaemia.

VIRUSES are associated with leukaemogenesis in both laboratory and outbred animals. In the laboratory mouse, susceptibility to several strains of virus leukaemia appears to be inherited and to involve an impaired immunological response to leukaemia specific antigens¹. These animal models are intellectually satisfying, and it is tempting to extrapolate to human leukaemias. Indeed, the current interest in immunotherapy to some extent presupposes that putative human leukaemia antigens are capable of inducing an immune response. Although Mathé and his colleagues² claimed that remissions in acute lymphoblastic leukaemia had been maintained with immunotherapy, they could find little difference between the duration of remissions induced using Pasteur BCG alone, using injections of allogeneic leukaemia cells alone or a combination of both methods. The British "Concord" trial³ using immunotherapy with modest doses of Glaxo BCG and without leukaemia cells failed to demonstrate any convincing advantage compared with maintenance chemotherapy. Others⁴ have, however, claimed success with active immunization in leukaemia and results using irradiated allogeneic myeloblastic cells are encouraging⁵. Similarly, prolonged remissions in acute leukaemia seem to be "spontaneous" following chemotherapy⁶ and might be due to the fortuitous development of effective immunity to leukaemia antigens.

In this article the usual abbreviations will be used—acute myeloid leukaemia (AML), acute lymphoblastic leukaemia (ALL), chronic myelocytic leukaemia (CML) and chronic lymphatic leukaemia (CLL).

Leukaemia cells compete successfully with their normal equivalents as a result of poorly understood metabolic adaptations, and it is unlikely that human leukaemia results simply from immunological failure. Thus it has recently been demonstrated that primitive cells obtained from patients with AML can be induced to normal differentiation by soluble substances secreted by mature granulocytes^{7,8} although it is possible that normal clones included with malignant cell suspensions are stimulated. These substances seem to be reduced in quantity in leukaemics⁹ and this suggests that AML could be the result of a deficiency of a granulocyte "maturation factor" analogous to the colony stimulatory factor (CSF) described by Metcalf¹⁰.

Metabolic alterations in leukaemic cells almost certainly involve alterations in cell membrane antigens, an idea which is supported by the suggestion in the hamster polyoma system that concanavalin A agglutination sites are related to malignancy and to the loss of cell contact inhibition¹¹. Such membrane alterations, although incidentally antigenic, may be more important in indicating altered malignant cell growth leading to escape from effective control (Fig. 1). Remissions maintained with immunotherapy do not necessarily imply the existence of specific leukaemia antigens, and it is possible that the kinetic advantage enjoyed by leukaemia cells relative to normal leucocytes might be temporarily negated by intensive non-specific stimulation of normal leucocytes, perhaps associated with increased levels of CSF.

Two essentially different types of human leukaemia antigens may be postulated. The first type, involving qualitatively new antigens associated with virus infection (or genetic mutation), would be immunogenic and theoretically could be used for active immunotherapy. The second type, involving quantitative variations of normal antigens, would be less likely to be immunogenic. The definition of antigenic changes characteristic of leukaemia is therefore important for a variety of reasons. First, their immunogenicity and value in immunotherapy could be evaluated and the course of immunotherapy could be monitored with specific immunological tests permitting the assessment of the relative value of allogeneic and autochthonous leukaemic cells. It is vital to know whether the same antigens are found in different patients with the same form of leukaemia, and whether specific immunological tolerance is involved in leukaemia. Second, the characterization of leukaemia antigens might allow the identification of human leukaemia viruses not so far detected by electron microscopy; and third, irrespective of the immunogenicity of leukaemia cell membrane changes a study of these might assist an understanding of the nature of the metabolic and kinetic changes induced by oncogenes.

Antibody Response to Leukaemia in Man

Antibodies reacting with leukaemia material have been reported in the serum of both leukaemia patients and normal individuals. For example, Seligmann *et al.*¹² detected precipitating antibodies in eight out of forty-five patients with acute leukaemia and granulopoena. These antibodies gave strong

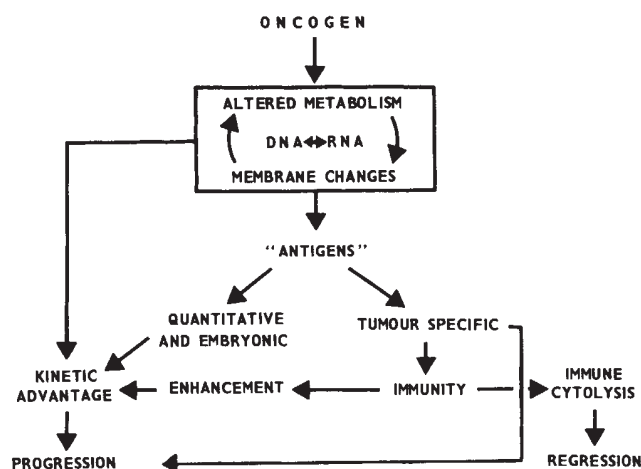


Fig. 1 Malignant transformation. Genetic changes following oncogenesis lead, it is suggested, to metabolic and membrane alterations which presumably confer kinetic advantages on malignant cells compared with their normal counterparts. "Tumour specific" antigens, but not quantitative variations in normal allo- and embryonic antigens, might be expected to lead to specific immunity and susceptibility to immune surveillance. Circumvention of the immune response might result from tolerance, antibody blockage or enhancement or non-specific immune depression.

reactions with ultrasonic lysates of both normal and CML leucocytes but not with CLL leucocytes. In a parous woman with acute monocytic leukaemia, Killmann¹³ detected an auto-antibody which, after absorption on autochthonous leukaemia cells and subsequent elution, reacted specifically with leukaemia cells. Garb *et al.*¹⁴ claimed to have obtained indirect evidence for the presence of auto-antibodies in gel diffusion studies in CML (see below). Greenspan *et al.*¹⁵, using passive cutaneous anaphylaxis in guinea-pigs and gel diffusion, found antibodies in the serum of most laboratory workers involved in human and animal leukaemia research. Greenspan also claimed to have induced similar antibodies by deliberate immunization of volunteers with human leukaemia brain material. Further studies using immunofluorescence suggested that the antigen responsible was located in the cytoplasm of leukaemia cells, and cross reactivity was reported between human and mouse leukaemia material. Using gel diffusion, immunoprecipitation and cytotoxic techniques, Carvalho¹⁶ detected auto-antibodies in all five leukaemia patients that he tested (four ALL and one acute stem cell leukaemia). Similar antibodies were found in 92% of 180 normal people. The antigen used was a purified protein extract of a pool of cells from fifty leukaemia patients. Using complement fixation, Armstrong *et al.*¹⁷ reported antibodies in 60% of healthy American adults when their serum was tested against lysates of leukaemia and of Burkitt's cell lines. Doré *et al.*¹⁸ found evidence for auto-antibodies in patients with several different forms of leukaemia. Four different techniques (cytotoxicity, immunofluorescence, mixed cell agglutination and complement fixation) only yielded positive tests for twelve of fifty-one patients, and in most cases only one of the tests was positive. Yoshida and Imai¹⁹, however, used immune adherence and studied untreated patients with various forms of acute and chronic leukaemia; they found auto-antibodies in fifteen out of thirty cases. Antibodies apparently increased in remission, and reactions with allogeneic leukaemic cells suggested the existence of at least two leukaemic specificities.

Cell Mediated Immune Response

Several attempts have been made to demonstrate cell mediated immunity to viable leukaemia blast cells obtained in the acute phase. Leukaemia cells can be inactivated after storage in liquid nitrogen and used in mixed lymphocyte culture (MLC) with autologous remission lymphocytes, thereby avoiding allogeneic differences. In many cases remission lymphocytes undergo blastoid transformation and are stimulated to DNA production by inactivated autochthonous blast cells although the response varies from patient to patient²⁰⁻²². Furthermore, after therapeutic injections of irradiated autochthonous or allogeneic leukaemia cells, the reactivity of suspensions of remission lymphocytes against active phase autochthonous blast cells appears to be increased^{20,22}, although the inclusion in cell cultures of autologous serum containing inhibitors (see below) and rebound following chemotherapy may lead to misleading results. The increased response was reported to be greater when autochthonous rather than allogeneic blasts were used to stimulate remission lymphocyte suspensions²². Leventhal *et al.*²³ also observed stimulation of remission lymphocytes by autochthonous blast cells and, in addition, reported *in vitro* cell mediated cytotoxicity against leukaemic cells and parallel cutaneous hypersensitivity to blast cell membrane. Such cutaneous hypersensitivity has been shown to correlate with remission^{23,24}. Cytotoxicity was also studied by Rosenberg *et al.*²⁵, who incubated lymphocytes from apparently healthy relatives and from normal controls with lymphoid cells, labelled with isotopes, from leukaemia patients and their normal identical twins. In four of six families studied, lymphocytes from family members were cytotoxic for lymphoid cells from a patient, but not from the identical twin.

These studies^{20-23,25} suggest that leukaemia cells have sur-

face antigen determinants to which immunological reactivity exists and which can be increased by immunotherapy. Rudolph *et al.*²⁶ failed, however, to find evidence for leukaemia associated antigens using mixed leucocyte cultures and testing four sets of identical siblings where one member of the set had acute leukaemia in relapse. In no instance did the leukaemia cells of the patient stimulate the lymphocytes of the normal twin. By contrast Bach *et al.*²⁷ investigated siblings, who, although not identical twins, were HL-A identical, and detected unidirectional stimulation which they interpreted as evidence for leukaemia associated antigens. The failure of Rudolph to detect stimulation in MLC might be ascribed to the lack of immunization in the normal twin, but it is then difficult to understand why Bach detected a mixed lymphocyte response in rather similar circumstances unless other non-HL-A antigens contribute to the mixed lymphocyte response between non-identical siblings.

Knight *et al.*²⁸ claim that established cell lines stimulate both allogeneic and autologous lymphocytes in culture, whether the cell lines are established from normal or leukaemia material, and they suggested that established cell lines develop membrane antigens not found on autologous normal lymphocytes. It was implied that the response of lymphocytes to autochthonous leukaemia cells was due to similar surface antigens and not necessarily to leukaemia specific antigens. The presence of "new" antigens on cell lines, however, in no way invalidates the evidence that leukaemia blast cells have specific antigens. The results of Knight and her colleagues emphasize the importance of the putative cell membrane changes which accompany altered cell kinetics—in this case those changes involved in adaptation to the abnormal condition of established culture.

In summary, the existence of leukaemia antigens in man has been suggested by immune reactions to autochthonous and allogeneic leukaemia material using a variety of techniques. These include the detection of antibodies by immunoprecipitation, leucoagglutination and mixed cell agglutination, immunofluorescence, serological cytotoxicity, complement fixation and passive cutaneous anaphylaxis in guinea-pigs. Cell mediated immunity has been detected by lymphocyte stimulation (MLC) and cytotoxicity *in vitro* and by cutaneous hypersensitivity against leukaemia material. It is not known whether the various forms of immunity that have been demonstrated inhibit or enhance the evolution of leukaemia. The variations in the strength and frequency of the immune reactions observed probably result from the different test systems and antigenic preparations used and the heterogeneity of the clinical types and stages of leukaemias involved. Two aspects of most studies are particularly poorly defined. The first involves the identification of leukaemia antigens. The second, which will be considered later, concerns possible variations in the level of general immune responsiveness at various stages in leukaemia.

Characterization of Leukaemia-associated Antigens

There have been many attempts, using xenogeneic antisera, to define specific antigens in leukaemia, although antigens defined in this way are not necessarily those that might provoke auto-immunity. Seligmann²⁹ claimed to have demonstrated antigens characteristic of CML in acute crisis. He immunized rabbits with whole human leukaemia cells and, in tube and gel immunoprecipitation, tested the resulting antisera against extracts of leukaemia leucocytes and serum from leukaemia patients. One antigen, apparently an α globulin, was found in the serum of both normal and leukaemia patients but, in acute relapse, a number of additional antigens appeared in the serum of patients with CML. Carvalho³⁰ employed rabbit antisera and purified fluoro-carbon extracts of protein from human leukaemia cells; he found extra precipitation lines with leukaemia material and noted that these antigens were different in CLL and acute stem cell leukaemia.

Korngold *et al.*^{31,32} studied the insoluble sediment from homogenized normal and leukaemia leucocytes with rabbit antisera and gel diffusion. They found that normal cells and CML leucocytes, by contrast with CLL, were good immunogens. They claimed to have defined three classes of antigens: antigens 1 and 2 ("granulocyte antigens") were found in normal leucocytes, in CML and in leucocytes from patients with leukaemoid reactions. Antigen 1 was reduced or absent in blast cells, which by contrast contained antigen number 3, which was also found in normal spleen and liver material. Antigens 1, 2 and 3 were absent from some acute leukaemic, from CLL cells and from two established leucocyte lines.

Garb *et al.*¹⁴, using rabbits rendered partially tolerant to normal antigens, raised antisera to whole blood from human leukaemia patients. In gel diffusion studies they detected antigens in AML which were not found in normal buffy coat. Antisera raised against CML blood reacted with both AML and CML but not with normal buffy coat material. CML leucocytes did not, however, react in gel diffusion with rabbit antisera, and Garb suggested that this was due to auto-antibodies coating CML cells. Also using antisera raised in rabbits rendered partially tolerant to normal human antigens, Hyde *et al.*³³ claimed to have detected antigens common to AML and CML and not found in normal leucocytes or in cells obtained from patients with CLL.

Yata *et al.*³⁴, using rabbit antisera raised against homogenates of lymph node cells from a patient with acute lymphoblastic leukaemia, detected cross reactivity in gel diffusion studies with most leukaemias, lymphomas and with some spleens and lymph nodes from normal individuals. Thymic cells obtained from normal individuals and fetuses (3 months gestation and later) were also positive. Yata believed that the antigen detected was similar to that of Tallberg *et al.*³⁵, and may represent a human thymus antigen comparable to mouse TL. Yata suggested that the thymic antigen on myelogenous leukaemia cells might be acquired during the passage of these cells through the thymus.

Mann *et al.*³⁶ prepared antisera by immunizing rabbits with purified cell membrane components from a tissue culture cell line, Raji (derived from a patient with Burkitt lymphoma). These sera, which were not absorbed before use, were found to be cytotoxic to peripheral white blood cells from some patients who had ALL or AML in relapse and not to cells from normal unrelated individuals. Evidence was, however, obtained which suggested that the antigenic determinants were present on white blood cells from some apparently normal relatives of leukaemia patients and from all the long term lymphoid tissue culture cell lines that were tested but not PHA transformed lymphocytes.

We have prepared solubilized partially purified extracts from haematologically defined human leukaemias³⁷⁻³⁹. The methods used were derived from those employed by Davies *et al.*⁴⁰ to extract membrane antigens from normal human spleens. Antisera raised in rabbits against these leukaemia materials react in gel diffusion and cross-over electrophoresis with both normal and leukaemia material. If, however, these antisera are absorbed with normal human serum and with extracts of normal spleens, reactivity with the normal material is greatly reduced. Absorbed antisera retain the ability to precipitate both leukaemia extracts and similar extracts made from normal embryos. Antisera prepared against material from patients with AML give strong precipitation lines with the serum of patients with AML or CML, but only occasional weak reactions with serum from patients with CLL or ALL and from normal individuals.

Problems and Pitfalls in Characterization

There are many variables in reported studies of leukaemia antigens. In some reports the haematological type and stage of leukaemia are not made clear. The methods for extracting antigens for immunization vary greatly, and a clear distinction

is not always made between water soluble and water insoluble fractions. In these circumstances considerable difficulty will be encountered as water soluble fractions of granulocytes strongly precipitate with extracts of other cells or with dialysed normal human serum³¹. Antisera produced against a mixture of antigens may contain antibodies with different optimal zones of reactivity, and one antigen may form a precipitation line whereas for another the ratio of antigen and antibody concentrations is unsuitable. Antigens which are good immunogens when injected into animals are not necessarily the most important antigens in distinguishing between different cell types.

Cross reactions between normal leucocytes and CML cells are reported in many studies, and are of unknown significance. Antisera raised against leucocytes contaminated with serum will react with plasma proteins in gel diffusion. Even when well washed leucocytes are used and antisera are absorbed with normal human serum, problems may arise because there is a wide range of human specific antigens against which rabbit antisera may inadvertently be raised⁴¹. Determining the correct method for absorbing unwanted specificities is often laborious and minor technical variations may produce anomalous results. Abnormal serum proteins which are not leukaemia specific may also cause difficulties in interpretation. For example, C-reactive protein (presumably the result of tissue breakdown) is found in the serum of over half the patients with acute leukaemia and can be demonstrated easily by gel diffusion studies⁴². Similarly a foeto-protein ($\alpha 2H$) found in large quantity in foetal liver appears in the serum of patients with many different blood diseases including leukaemia⁴³. The distinction between such non-specific antigens as C-reactive protein and $\alpha 2H$, on the one hand, and "true" tumour antigens is, however, blurred, for it is known that carcino-embryonic antigens⁴⁴ and $\alpha 1$ foeto-protein⁴⁵, previously thought to be pathogenic for particular malignancies, are present in minute quantities in normal individuals (see later).

Variations in Normal Iso-antigens

Several variations in leucocyte allo-antigens have been described in leukaemia. For example, Kassulke *et al.*⁴⁶, using mixed agglutination, claimed that there occurs in some acute leukaemias a reduction in group A and H antigenic specificity together with the appearance of the same M and N antigens which are found on the patient's red cells. These authors stated that M and N antigens are not normally demonstrable on leucocytes, and account for the reduction in A and H activity by the acquisition by leukaemia cells of neuraminic acid with M and N specificity.

Variations in HL-A antigen expression in leukaemia have also been reported. Pegrum *et al.*⁴⁷ claimed that leukaemia cells may acquire new HL-A antigens. "Additional" HL-A antigens have been observed in lymphoid cells in culture^{48,49}. We have suggested⁵⁰ that anomalous HL-A typing results on occasions from the contamination of HL-A antisera with antibodies too weak to be detected by cytotoxic tests employing normal cells. For example, low titre EB virus antibodies are detectable in almost 50% of normal individuals⁵¹. We find that AML blasts in common with established cell lines are more sensitive to HL-A antisera. Although shorter incubation times and a lower concentration of rabbit complement may yield clear results, some HL-A antisera continue to give anomalous results with leukaemic cells suggesting that they contain antibodies directed against antigenic determinants not demonstrable on normal lymphocytes.

Goret *et al.*⁵² claimed that lymphocytes from patients with acute leukaemia were lysed by a wide range of xenogeneic and allogeneic antisera in circumstances in which normal lymphocytes are not, and that the lymphocyte cell membrane in leukaemia is abnormally fragile. In a small series of children with ALL we have not detected increased lymphocyte sensitivity to cytolysis by HL-A antisera (Harris, R., and Wentzel,

J., unpublished observations). Schlesinger and Amos⁵³ believed that leukaemic cells were generally more reactive than normal cells with allo-antisera.

It is interesting to contrast the apparent gain of HL-A antigens with the phenomenon of antigenic modulation in mice^{54,55}. TL ("thymus leukaemia") antigens are normally present on thymic cells in "TL positive" strains of mice, and may appear in the peripheral blood when TL positive or TL negative mice develop leukaemia. The appearance of TL on blood cells is associated with a quantitative diminution of normal H-2 histocompatibility antigens. If TL positive cells are injected into specifically immunized TL negative mice⁵⁴ or incubated with non-cytotoxic TL antisera *in vitro*⁵⁵, TL antigenicity decreases and H-2 antigenicity increases. Antigenic modulation thus involves a reciprocal relationship between H-2 and TL. Although no equivalent phenomenon has so far been discovered in human leukaemia, Martin *et al.*⁵⁶ have reported that a normal antigen diminishes in quantity in parallel with the increase in carcino-embryonic antigen in colonic tumours. Quantitative reduction in the expression of normal antigens may be a rather common phenomenon accompanying tumour antigens and human leukaemia cells should be re-examined with more discriminating techniques, and making allowance for possible membrane fragility, to detect a reduction in the expression of HL-A antigens.

Quantitative antigen changes may also result from alterations in the proportions of different circulating leucocyte types. In CLL, for example, cells tentatively identified by immunofluorescence as "B" cells make up more than 85% of the peripheral blood lymphocyte population compared with 34% in normal individuals⁵⁷. These studies show that an increase in total immunoglobulin receptors occurs due to an increased number of "B" cells rather than an increased density of immunoglobulin receptors on the cell membrane. Very little is known about the spatial distribution of antigens on the surface of human leukaemia cells. Using the phenomenon of steric interference Legrand and Dausset⁵⁸ have, however, begun to map the position of HL-A antigens on normal lymphocytes, and Aoki and his colleagues⁵⁹ studying mouse cells have demonstrated the existence of an antigenic "grid" in which a number of antigens characteristic of thymus derived lymphocytes exist in a definite spatial relationship with each other. Although these observations have been criticized by Davis *et al.*⁶⁰, it is reasonable to hope that a study of human leukaemia cells will clarify some of the changes which are suspected to occur provided leukaemia associated antigens can be accurately defined and effectively monospecific antisera to these antigens raised.

Embryonic Antigens

Our results³⁹ indicate that cross reactions occur between solubilized partially purified extracts of leukaemia cell membranes and extracts prepared in a similar way from normal human embryos of about 12 weeks gestation. A limited number of experiments suggest that the antigens concerned is not $\alpha 1F$. $\alpha 2H$ occurred in more than half of the patients with AML, but could be distinguished from other antigens of solubilized leukaemia cell membranes. In studies in collaboration with Professor Berg of Oslo we compared an anti-AML serum with rabbit antiserum, raised against human amniotic fluid, which did not react with $\alpha 2H$. These antisera gave reactions of identity in immunoprecipitation and very similar reaction patterns ($P < 0.001$) with a panel of sera from patients with leukaemia. Others⁶¹ have also found evidence for the existence of embryonic antigens in human leukaemia material.

The reappearance in cancer of antigens characteristic of the foetus is now known to occur in many different human and animal tumours⁶². This has been variously ascribed to the specific derepression of genes important in embryonic development (perhaps indicating a reversion to an uncontrolled embryonic pattern of cell growth), to the persistence of small

numbers of "embryonic cells" or to stem cells in adult tissues and to a general disruption of normal gene control (perhaps associated with chromosomal aneuploidy) allowing the random expression of many genes normally repressed in adult cells.

Embryonic antigens, notably $\alpha 1F$ and CEA, have been shown to occur in nanogram quantities in normal adult tissues and plasma^{44,45}, and it is not clear to what extent these antigens might be auto-immunogenic in cancer patients. This is a very important question because *a priori* specific immunotherapy would be more likely to succeed if qualitatively new (tumour specific) antigens are involved. Nanogram quantities of embryonic antigens in normal adult serum may, however, not be sufficient to maintain tolerance, for recent work⁶³ with immunoglobulin idiotypes suggests that an immune response can be mounted against antigens previously present in sub-tolerogenic concentrations.

Surface Charge

It is believed that the affinity of antibodies for cellular immunogens is governed by an inverse relationship between the relative net electrical charges⁶⁴, and Ambrose⁶⁵ has suggested that cancer cells have increased surface negativity. Sahasrabudhe *et al.*⁶⁶ claimed to have produced antibodies "specific" for leukaemic cells by immunizing rabbits with normal leucocytes which were previously tagged with 1-fluoro-2,4-dinitrobenzene (FDNB). They ascribe this phenomenon to alterations in net surface charge induced by FDNB. Others, however, have not found differences in surface charge in unmodified ALL cells⁶⁷.

Association between HL-A and ALL

Walford⁶⁸ reported a significant excess of HL-A2 and HL-A12 antigens in children with ALL, and suggested an analogy with Gross virus induced mouse leukaemias where inbred strains of H-2^{kk} genotype are susceptible to specific oncogenic virus whereas H-2^{dd} mice are resistant. Mice which develop leukaemia due to Gross virus may lack a "resistance gene" (Rgv-1) located within the H-2 chromosomal region¹. The demonstration of comparable associations between susceptibility to leukaemia and histocompatibility type in man would provide important evidence favouring the similarity between murine and human leukaemia and strengthening the hypothesis that human genetic susceptibility to oncogenic viruses is involved. By analogy with experimental animals, particularly the mouse, the HL-A chromosomal region may contain genes controlling susceptibility to leukaemia and other diseases and the levels of specific and general immune response to various antigens (reviewed by McDevitt and Bodmer¹ and Walford *et al.*⁶⁹). Susceptibility to leukaemia may be determined by recessive mutant genes governing a poor immune response to leukaemia (virus) antigens. Alternatively, the antigenic products of co-dominant genes in the HL-A chromosomal region may be so similar to leukaemia or virus antigens that individuals positive for such antigens may be unable to produce an immunological response to them. Unfortunately the genetic heterogeneity of human populations makes the search for associations between genetic markers and diseases extremely difficult⁷⁰.

Viruses and Leukaemia

It is likely that at least some human leukaemias will be shown to be associated with oncogenic virus, although it would be inappropriate to attempt to detail the evidence here. Virus particles have not been found consistently in leukaemia in man⁷¹, but the antigens of tumours and leukaemias in animals have been reviewed⁷² as has the evidence for the involvement of viruses in human cancer⁷³. RNA viruses seem to manufacture DNA in mammalian cells by means of reverse transcriptase, and it has been suggested that such DNA may be incorporated into the mammalian genome and transmitted in the germ line⁷⁴. Work with feline viruses⁷⁵ has provided a model in which RNA oncogenic virus is probably transmitted

vertically and horizontally in outbred animal populations overcoming the objections frequently raised against the relevance of leukaemia of inbred mice. Cat leukaemia virus, like other C-type RNA viruses, can be characterized by type and group specific antigens using allogeneic and xenogeneic antisera. Interspecies antigens, which appear to be common to virus leukaemias affecting cats, rats, mice and hamsters, may, however, prove to be more valuable in the search for leukaemia viruses in man.

Spiegelman has shown that RNA from Rauscher mouse virus leukaemia can be used successfully to detect homologous nucleoprotein in human leukaemia⁷⁶. Furthermore, his group has demonstrated complexes of 70S RNA and reverse transcriptase in the white blood cells of 95% of leukaemic patients examined. DNA synthesized by these complexes hybridized to the RNA of Rauscher leukaemia virus but not to other animal tumour viruses studied. Such complexes were found in patients with ALL, AML and CML⁷⁷.

Depression of the Immune Response in Leukaemia

I have suggested in this article that there is evidence, albeit fragmentary, for specific antigens in human leukaemia. Why is the immune response to these antigens apparently ineffective? A general depression of immunity has not been conclusively demonstrated in untreated acute leukaemia⁷⁸⁻⁸⁴. Much attention has, however, been concentrated on the *in vitro* lymphocyte response to phytohaemagglutinin (PHA) in an attempt to overcome the variables inevitably associated with the evaluation of immunity. Most studies have shown that the PHA response is relatively normal in patients with untreated acute leukaemia, and although some believe that the response is depressed by chemotherapy it recovers rapidly when treatment is stopped. The dose of PHA used in many studies is, however, much in excess of that which gives the most sensitive discrimination between normal and abnormal responses^{85,86}. We have found⁸⁷ that the response of lymphocytes from normals or leukaemics to low ($7 \mu\text{g ml}^{-1}$) doses of PHA is often greatly reduced in the presence of serum obtained from patients with untreated AML and ALL (Fig. 2). We attribute this to the presence of inhibitory factors in the serum of leukaemics only occasionally attributable to complement dependent cytotoxic HL-A antibodies.

Hersey *et al.*⁸⁸ detected blocking factors to antibody dependent *in vitro* killing of leukaemic blast cells by lymphocytes. In one patient of the small series studied so far relapse was preceded by the appearance of "blocking factors" and with the appearance of our inhibitory factors. The *in vivo* importance of antibody dependent lymphocyte killing of leukaemic cells and the role of blocking factors are currently

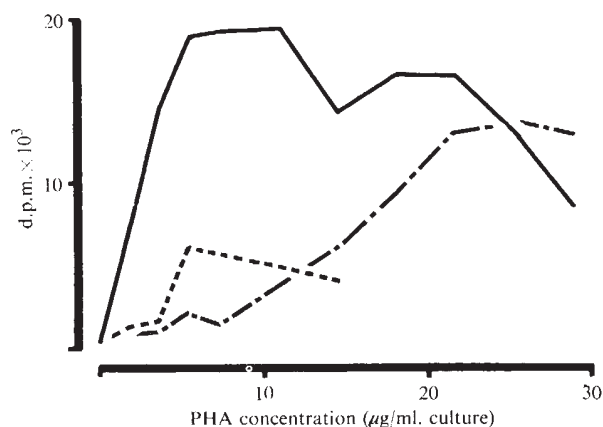


Fig. 2 Inhibitory effect of leukaemic serum on ^{125}I udr uptake by PHA stimulated lymphocytes from a normal donor. —, Normal autologous serum; ---, serum from untreated ALL; ·····, serum from untreated AML.

being assessed. It may eventually become clear that the Hellström model⁸⁹ of antibodies blocking cytotoxic lymphocyte activity against tumour cells is also valid for leukaemia.

Serum inhibitors of the lymphocyte response occur in many different diseases⁹⁰, although their immunological significance is unknown. Field and Caspary⁹¹, using washed lymphocytes, claimed, however, a normal *in vitro* response to PPD in patients with sarcoidosis, although the Mantoux test was negative and the addition of a 1 in 60 dilution of autologous serum abolished the *in vitro* response to PPD. Our results demonstrated that leukaemia sera frequently inhibit two-way mixed normal lymphocyte reaction as well as the PHA response. This suggested that serum inhibitors have *in vivo* immunological significance and perhaps interfere with the function of otherwise normal lymphocytes. Serum factors may also inhibit normal haematopoiesis and consequently alter the kinetic balance between leukaemia and normal cells (Lajtha, personal communication).

Immune Phenomena

The current preoccupation with immunotherapy is based on the assumption that potentially immunogenic changes occur during leukaemogenesis. Many antigen alterations in leukaemia are, however, not necessarily immunogenic; for example, an increase of embryonic or normal allo-antigens and the vertical transmission of a leukaemia agent may be associated with immunological tolerance. Nevertheless the intellectual appeal of immune surveillance is so great that one is almost compelled to believe that immune phenomena are involved in leukaemia and a review of the literature indeed suggests that potentially immunogenic antigenic changes characteristic of human leukaemia occur. Thus auto-antibodies have been reported at various stages in the evolution of leukaemia, antibodies to leukaemia cells have been described in the serum of normal individuals who have been in contact with human or animal leukaemia and peripheral lymphocytes respond *in vitro* to autochthonous leukaemia cells in a way that suggests that immunogenic leukaemia associated antigens are present. If auto-immunogenic leukaemia associated antigens exist, it must be supposed that the results of immune response are ineffective in established leukaemia.

The serum of patients with untreated acute leukaemia is frequently inhibitory to lymphocytes both in the PHA and the mixed lymphocyte responses. These factors inhibit allogeneic and autochthonous lymphocytes from leukaemia patients and normal individuals alike. Some inhibitors may be α globulins⁹², and we have observed a marked fall in both inhibitors and $\alpha 2\text{H}$ levels following the induction of remission in AML (unpublished observations). It is not clear whether the presence of inhibitors is merely incidental or whether they play an important part in suppressing normal immunity and haematopoiesis.

Under the aegis of the MRC a trial of immunotherapy in AML is now in progress. This trial includes a programme of investigations designed to study both specific and non-specific immunological responses. An important object of the trial will be to evaluate the relationship between the afferent (recognition) and efferent (cytotoxic) arms of immune responsiveness in leukaemia and to study the effects of immune depression, whether endogenous or due to chemotherapy, on putative leukaemia specific responses.

There is almost certainly no single cause for leukaemia, and individual susceptibility is probably governed by a complex interaction of oncogen and inherited host resistance. The concept of immune surveillance does not require that immunity should have a primary role in the inception of cancer and the fundamental change is the imperfectly understood metabolic adaptations of malignant cells which allow them to compete successfully with their normal counterparts. There is, however, evidence for auto-immunogenic antigens associated with human leukaemia, and it will probably be shown that in some cases these result from infection with oncogenic virus. A

knowledge of such antigens would allow an aetiological classification of human leukaemia and would provide a rational basis for successful immunotherapy following cyto-reductive chemotherapy.

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Premonitory Changes in Seismic Velocities and Prediction of Earthquakes

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Large premonitory changes in ratio of seismic P and S velocities correlate with time of occurrence and size of earthquakes at Blue Mountain Lake, New York. These changes may be related to the opening of cracks or to changes in pore pressure.

WE report here large changes in the ratio of compressional to shear wave velocities (v_p/v_s) in the source region of several small to moderate size earthquakes in the eastern United States. Although our results are only preliminary and many additional data remain to be analysed, the changes we observe are pronounced and are similar to observations reported for the Garm region of Central Asia^{1,2}. To our knowledge this is the first time that the work in Garm has been repeated successfully elsewhere. We hope these preliminary observations will encourage the search for similar phenomena premonitory to earthquakes in other areas.

A series of small to moderate size earthquakes, the largest of which was magnitude 3.6, in the Adirondack region of northern New York State (Fig. 1) in 1971 provided an unusual opportunity to install a network of portable seismographs within a few kilometres of the source region³. Because the focal depths of the events in this swarm were between 0 and 3.5 km, a large percentage of the seismic ray paths were within the volume defined by the spatial distribution of the shocks in the swarm.

Much of this report seeks to document that large and significant changes in the P to S velocity ratio do, in fact, occur and that this ratio decreases prior to the larger shocks that we studied. This decrease, which is up to 13%, is similar to premonitory changes before magnitude 4 to 5 earthquakes in Central Asia. The time duration (but not the size) of the anomalous decrease in velocity ratio is longer the larger the magnitude of the earthquake. Although we do not understand the causal mechanism for these large changes in velocity ratio, we discuss several factors such as changes in pore pressure, crack development and creep that may cause these large anomalies in velocity ratio. Detailed monitoring of the velocity ratio and an understanding of the causes of these changes may provide sound bases for predicting earthquakes in general or for ascertaining the type of earthquake or seismic area for which this technique is applicable.

Description of Swarm

Six portable high-gain, high-frequency seismographs were installed (Fig. 1) near Blue Mountain Lake (BML) a few days after two events of magnitude 3.6 and 3.4 which occurred on May 23, 1971. Observations of the swarm, which consisted of thousands of recorded microearthquakes, continued

for several months. Events as large as magnitude 3.3 were recorded by the portable network. We report here the velocity ratio determined from small events before and after the July 10 and 27 events of magnitude 3.3 and 2.5.

Sbar *et al.*³ analysed the spatial distribution of events in the swarm and report a composite focal mechanism involving thrust faulting. The earthquake foci are confined to a tabular zone that dips gently eastward from the surface to a depth of about 2 km and then steepens for depths from 2 to 3.5 km. The surface projection of this zone is about 2 km on a side and is somewhat larger than that involved in the foreshocks and aftershocks of the July 27 event of magnitude 2.5 (Fig. 1).

v_p/v_s Technique

For each of several hundred small earthquakes the arrival times of P and S waves at each seismograph station were read with a precision of 0.01 s using a microscope. Onsets of both the P and S waves were very impulsive; problems of phase identification were negligible for the events reported here. S-P times for each shock were plotted as a function of P arrival times as illustrated in Fig. 2 for three earthquakes. The slope of these curves is the ratio of the travel time of S waves to that of P minus one. For a homogeneous material the slope is $v_p/v_s - 1$. The precise fit of the data points in Fig. 2 to straight lines attests to the accuracy of the readings of arrival times and to the homogeneity of the medium. One factor responsible for the small scatter of the observations

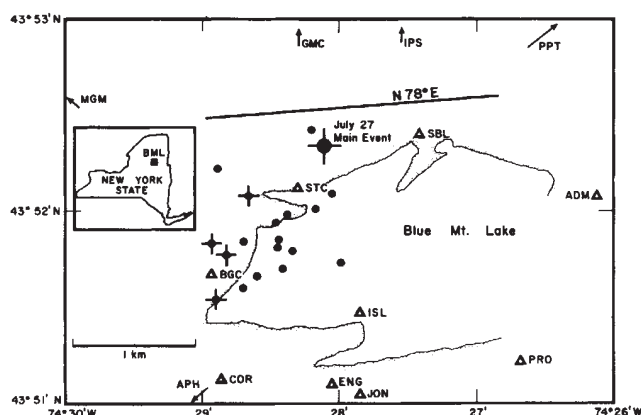


Fig. 1 Location of Blue Mountain Lake (BML) earthquake swarm. Solid circles are epicentral locations of small earthquakes that occurred within 3 days before and after magnitude 2.5 event of July 27, 1971. Bars indicate errors in epicentral locations; no bars denote locations with errors smaller than size of symbols. Notice that main event is at one end of the focal region. Triangles show seismograph sites, and arrows indicate direction of sites outside area of figure. N 78° E is a line perpendicular to the strike of fault plane as inferred from focal mechanism³ and is trend of vertical section in Fig. 6. Insert shows location of BML area in New York State.

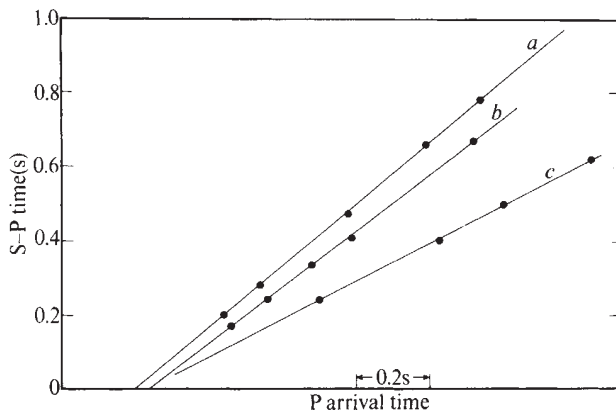


Fig. 2 S-P times as a function of P arrival times at various stations for three earthquakes. The slope gives $(v_p/v_s - 1)$ and the intercept (S-P)=0 is origin time of earthquake. Notice the small scatter (0.1 s or less) in data points from straight lines, and marked variation in v_p/v_s ratio. *a*, July 8 (1946), $v_p/v_s = 1.84 \pm 0.04$; *b*, July 27 (1971), $v_p/v_s = 1.77 \pm 0.03$; *c*, July 6 (1971), $v_p/v_s = 1.52 \pm 0.05$.

for single shocks is the near absence of surficial sediments; gneiss outcrops widely in this area. Fig. 2 demonstrates marked variations in the v_p/v_s ratio for the three events and gives the range of S-P times, 0.15 to 0.8 s (that is, epicentral distances of about 1.0 to 6.5 km).

Changes in v_p/v_s

Figs. 3 and 4 show individual determinations of v_p/v_s as a function of time before and after the July 10 and 27 events, two of the largest shocks in the swarm. Arrows indicate the time of occurrence and relative size of all events larger than magnitude 1. In both Figs. a pronounced, broad minimum of a few days duration in v_p/v_s occurs before each of the two largest earthquakes. From July 1 to 3 and July 11 to 12 (Fig. 3) during which period no shocks larger than magnitude 1 occurred, the velocity ratio does not change appreciably from 1.75 (a Poisson's ratio of 0.26). Similarly, the ratio did not change significantly from 1.75 over a 7 day period in August (Fig. 5) during which time no shocks larger than 1 occurred. The largest (1.9) and smallest (1.52) velocity ratios in Figs. 3 and 4 differ by 22%.

Fig. 3 Velocity ratio v_p/v_s as a function of time for series of small earthquakes before and after the largest event studied (July 10, 1971, magnitude 3.3). Vertical bars indicate $\pm$ s.d. in v_p/v_s measurement, arrows and numbers indicate the magnitude and time of occurrence of all events larger than magnitude 1 from July 1 to 12, 1971. Notice pronounced minimum in velocity ratio from July 4 to 8 that we tentatively associate with largest event on July 10; similar minima a few hours before smaller shocks on July 8 and 9 of magnitude 2.0 and 1.8; and the comparatively constant value of v_p/v_s before July 4 and after July 10 when no events larger than magnitude 1 occurred.

Fig. 4 Velocity ratio v_p/v_s as function of time before and after event of July 27, 1971, of magnitude 2.5. Symbols as in Fig. 3. Notice duration of minimum in v_p/v_s , which we associate with larger earthquake on July 27, is similar to that for event of magnitude 3.3 in Fig. 3. Minimum on July 29–30 may be related to event on July 30. No minima were detected before shocks on July 21, 22 and 24 but sampling may be too sparse to detect short fluctuations in v_p/v_s . Two anomalously high points on July 26 are associated with epicentres nearest the northwestern end of focal region in Fig. 1.

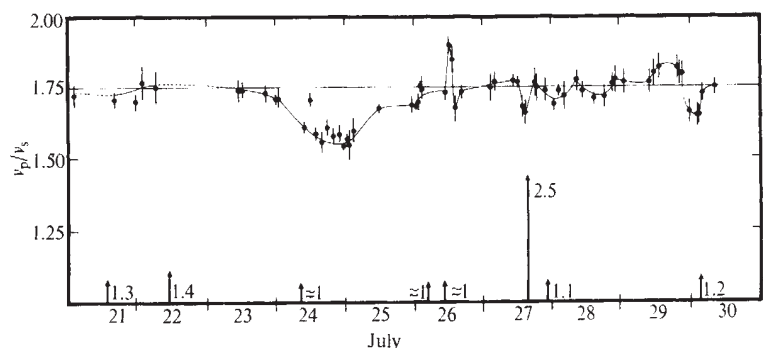
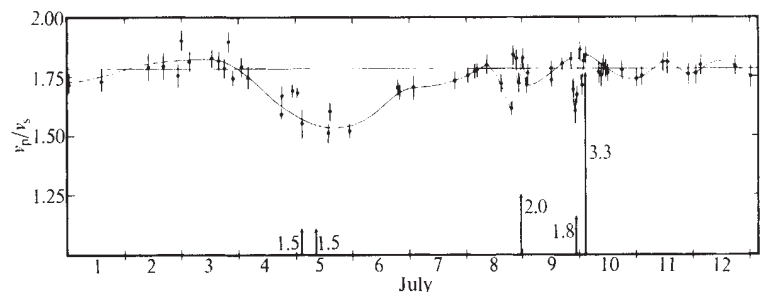
Pronounced minima of a few hours duration seem to precede some shocks as small as magnitude 1.2 (Figs. 3 and 4). Minima for two shocks of magnitude 1.8 on August 14 (not shown) are nearly identical to that of the magnitude 1.8 event of July 9 (Fig. 3). Although minima were not detected before several other shocks of magnitude 1 to 1.5 (Figs. 3 and 4), the sparse data sampling may have missed rapid fluctuations in v_p/v_s .

Fig. 6 shows a vertical section perpendicular to the strike of the fault plane as inferred from a composite focal mechanism solution³. The section indicates positions and depths of those small shocks in Fig. 1 that occurred within three days of the magnitude 2.5 shock of July 27. The v_p/v_s value beside each earthquake shows no apparent correlation with either depth or position; neither do they vary significantly with azimuth or station location.

Semonova² found that the time duration of a pronounced minimum in v_p/v_s before earthquakes in Central Asia was proportional to the magnitude but that the percentage decrease was not a function of magnitude. The time duration of the pronounced minimum in velocity ratio for our data and that of Semonova² increases with increasing magnitude (Fig. 7). It is not clear, however, whether magnitude is a linear function of time as Semonova proposes or of log time as Fig. 7 might suggest. In any case extrapolation to larger magnitudes gives durations of months or longer for events of magnitudes greater than 6. Using a magnitude-fault length (*L*) relationship derived for California earthquakes⁴ and assuming a linear relationship in Fig. 7 between log time and magnitude, we obtain time duration proportional to $L^{1.6}$.

Causal Mechanism

The earthquakes at BML and those in Central Asia for which large changes in velocity ratio were found are similar in several ways. Focal mechanisms are of the thrust type and the depth of focus is shallow. The depths of the events we studied were less than 2 km while most of those in Central Asia were less than 10 km. The seismic signals of BML events are rich in high frequencies. This may be indicative of high stress although efficient propagation alone may explain the enrichment of high frequencies. It is not unreasonable that thrusting related to continental collision in Central Asia may also be typified by high stress. Thus, it is not certain if similar changes in velocity ratio occur in other



areas of dissimilar tectonics or rock type or where the focal depths are not quite as shallow. Nonetheless, the tectonic style in other areas such as in south-central Alaska and the Transverse ranges of California is similar in many ways to that in the above two areas.

It is indeed surprising that changes as large as 13% in velocity ratio occur in such a short time before small to moderate size earthquakes. This time is very short compared with what is normally thought to be the period of strain build-up in the Earth but is long compared with that for seismic wave propagation. Thus, it seems necessary to appeal to phenomena such as pore pressure variations, fluid flow, crack formation or creep that have appropriate time constants.

Increased pore pressures are known to be associated with several natural and man-induced earthquakes. Nur and Booker⁵ suggest that aftershocks of natural earthquakes and of large underground nuclear explosions may result from a redistribution of pore pressure and fluid flow along faults. Assuming that fluid flow may be approximated by a diffusion equation, they obtain a time constant (τ) proportional to a characteristic length (L) squared. For $L=1$ km they obtain $\tau=1$ day for fractured and jointed rock. These values are comparable to the aftershock dimensions (Figs. 1 and 6) and to the time duration of minima in velocity ratio for BML

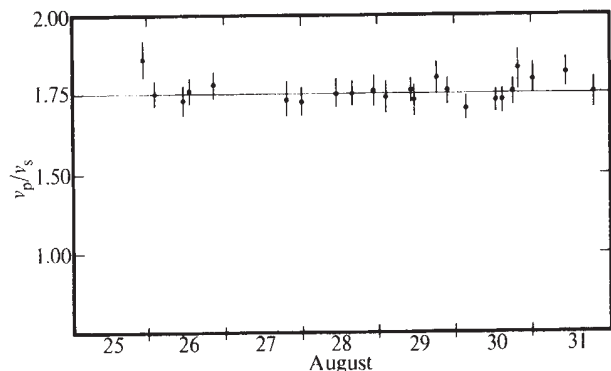


Fig. 5 Velocity ratio as function of time for 7 day period in August 1971 during which no earthquake of magnitude ≥ 1 took place. Symbols in as Fig. 3. Notice the absence of pronounced changes in v_p/v_s such as those in Figs. 3 and 4.

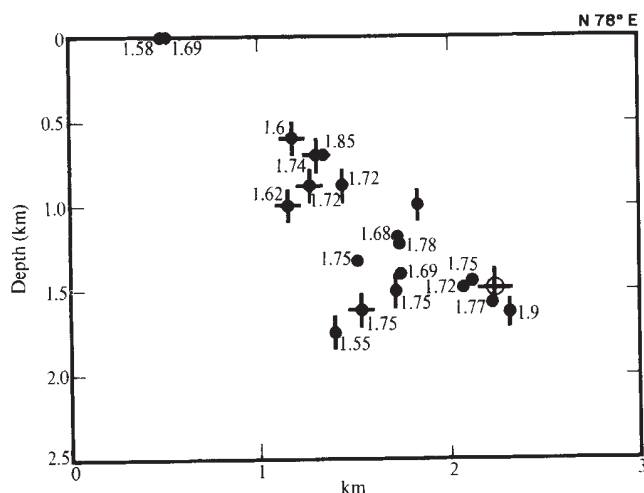


Fig. 6 Vertical section through hypocentral zone parallel to section line in Fig. 1. Data same as Fig. 1. Values of velocity ratio v_p/v_s shown beside solid circles that denote individual earthquakes. Bars indicate errors in location; no bars indicate errors were smaller than size of circles. Notice absence of any apparent correlation between the values of v_p/v_s and the focal depth. Note largest shock in this sequence (open circle) is located at down dip end of focal region.

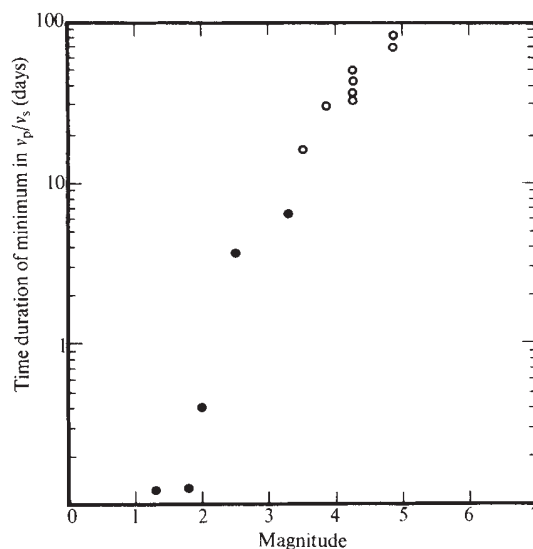


Fig. 7 Time duration of minimum in velocity ratio (measured from onset of minimum to time of earthquake) as function of magnitude. Solid circles are BML data and open circles are for Central Asia².

shocks of magnitude near 3. The exponent 1.6 obtained earlier in the relationship of time duration of the velocity ratio minimum and fault length is intermediate between that for solutions to the wave equation (1.0) and that for fluid flow by diffusion (2.0). Nason and King⁶ report creep events on the San Andreas fault that propagate with velocities of 0.5 to 6.0 km day⁻¹. Johnson *et al.*⁷ correlate water fluctuations in a well in the San Andreas fault zone with creep events. Thus, fluid pressure variations and propagation of creep satisfy the time constants and fault dimensions obtained for BML and Central Asian events.

It is interesting to note here that the two largest events in the swarm on May 23, 1971, occurred two days after an extremely heavy rainfall that resulted in a rise of about 25 cm in the level of BML³. Foreshocks, however, were observed well before the rainfall. Thus, it is unlikely that this very small pressure change could have acted as anything more than a "last straw".

Gupta⁸ reports small decreases in P and in S velocities before shear failure in laboratory tests. If P and S velocities both decrease or both increase but by different amounts, percentage changes in P or S much larger than 13% must be involved for BML events. Scholz⁹ reports microfracturing and dilatancy (decreased volumetric strain) accompanying crack growth in rocks deformed in laboratory experiments. These effects become very pronounced within a few percent of the fracture strength of the rock. The presence of cracks drastically reduces the P and S velocities of dry rocks for confining pressures (or effective stresses) below about one kbar¹⁰. When these cracks are filled with water, however, the P velocity is not reduced as drastically for low confining pressures, whereas the shear velocity still decreases markedly at low confining pressures and is nearly the same as in dry rocks¹⁰. Thus, pronounced and rapid changes in the P to S velocity ratio at low effective stress can arise from changes in effective stress caused by crack development or changes in pore pressure for saturated rocks; or the rock passing from a saturated to unsaturated environment or vice-versa. The second alternative, however, seems to be less probable for BML because nearly all of the earthquake foci are likely to be below the water table.

The premonitory minima observed at BML and in Central Asia consist of a decrease in velocity ratio followed by an increase before the earthquake. We tentatively associate the decrease in velocity ratio with the rapid growth of cracks

which leads to increased dilatancy, a drop in pore pressure, an increase in effective stress, and a more rapid increase in v_s than in v_p . The increase in velocity ratio is more difficult to explain but may be related to a subsequent increase in pore pressure as fluids either propagate along newly connected cracks or permeate into cracks from the surrounding medium. This model is consistent with Brace and Martin's¹¹ observation that strength is a marked function of strain rate for rocks of low porosity and permeability. Observations of changes in fluid pressure and perhaps of changes in radon exhalation (as observed before the Tashkent earthquake of April 26, 1966, see ref. 12) as a function of time should provide a check on our model.

With our present data we cannot ascertain whether changes in P, S or both velocities are responsible for the observed changes in velocity ratio. Nevertheless, the changes in v_p/v_s are large enough that small explosions should be useful in ascertaining how individual P and S velocities change and in checking the results obtained from earthquakes. Repeated explosions may provide a means of monitoring v_p/v_s and of predicting earthquakes in areas where it is not convenient or possible to use small earthquakes.

Very many more data from BML remain to be analysed. Some time after July 27 the maximum depth of earthquakes increased from 2.0 to 3.5 km. This apparently extended the rupture zone down dip to the east. The focal mechanism of these deeper events, while remaining of the thrust type, involved a significant change in the direction of maximum compressive stress compared to that for the shallowest shocks³. In a few cases the velocity ratio varies as a function of epicentral distance. We were surprised that this effect

was observed for only a few percent of the shocks analysed. This suggests that the epicentral distances of our stations were still close to the strained region for most events. Perhaps the most perplexing observation is that the percentage change in velocity ratio is nearly the same for large or small shocks even though we presume the rupture lengths are quite different in size. We plan to analyse these effects in more detail.

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Cyclical Changes in the Habitat and Climate of an East African Ecosystem

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Ecological changes in an East African Game Reserve may be attributed to changes in climatic conditions and soil salinity. Tree damage from elephants and overgrazing by livestock are probably secondary factors.

Maasai Amboseli Game Reserve has suffered extensive loss of the fever tree (*Acacia xanthophloea*) woodlands over the last two decades. The decline was accompanied by a marked shift towards a more arid habitat, with overgrazing by Maasai livestock frequently regarded as the principal cause¹. An alternative hypothesis links the changes to the elephant destruction problem, widespread in other East African Parks and Reserves, for example Tsavo, Murchison Falls and Manyara. Laws^{2,3} suggests that these elephant-induced habitat changes stem from the disruption of local populations caused by human encroachment. Here we describe the magnitude of causes of woodland and other habitat changes in Amboseli. We also demonstrate the need for a long-term perspective of

these changes, important to the future management of Amboseli and possibly habitat changes in other East African ecosystems.

The area concerned, the Amboseli basin, has been briefly described⁴ and is the site of a lake that formed during the Pleistocene and dried out in the Recent epoch⁵. Rainfall averages 350–400 mm per annum supporting a dry savannah, bushed grassland⁶. Fever tree woodlands which formerly covered much of the basin area until the mid-1950s are generally associated with a high ground water-table. To evaluate the extent of changes within the woodlands, thirty random 18 hectare plots were drawn on a mosaic of 1950 aerial photographs and replicated on 1961 and 1967 mosaics. The sign test showed a significant decline between each of the data points ($P < 0.001$) with an 11% loss from 1950 to 1961, reaching 70% by 1967. Allowing for a bias of counting dead standing trees which constituted approximately 60% of the standing trees in 1967, we estimated that some 90% of the woodland trees were lost over this period, largely in the last decade.

In seeking the causal agents of "die-off" we recorded on the ground the condition of 20 or more trees in 18 stands, together with the incidence and level of elephant damage. Condition was estimated from the ratio of dead or moribund to intact major branches. In each stand soil samples were

collected at 30 cm intervals down a profile 180 cm deep, or until the hardpan was reached. Textural and chemical analyses were obtained for each sample. The distribution patterns of Maasai settlements and livestock were available on a monthly basis over a 3 year period from 1967 to 1970⁴.

The progressive increase in the number of dead trees towards the centre of the basin was negatively correlated with the density of livestock (Fig. 1). Also the area of highest woodland mortality has been a wildlife sanctuary from which livestock have been excluded since 1961, before the major decline of trees. Similar areas around the Reserve headquarters at Ol Tukai have been stock-free since 1947. Conversely, the basin-edge woodlands which have suffered least loss have, for various reasons, been the areas of highest settlement and cattle density for decades, which does not support the hypothesis that livestock are responsible for the habitat changes.

Elephant damage is extremely conspicuous; over 83% of trees examined showed some degree of debarking, although less than 1%, mainly young trees, had been pushed over. Debarking might impair water transport in the conducting tissue and result in death through "ring barking"; this was observed in a few cases where most of the stem was debarked. No statistically significant correlations were found between either individual tree condition or general stand condition and the incidence of elephant damage ($P > 0.1$). A substantial contribution to the variance was probably due to differential regeneration in trees of different ages. By taking height class as an approximation to age class and plotting the mean condition of each against the incidence of elephant damage, it was possible to reduce this source of error. The relationship of elephant damage to tree condition then appears highly correlated ($r = 0.80$, $P < 0.02$). Two observations, however, lead to the conclusion that elephant damage is not the sole or even primary cause of death, but amplifies other causes of death. First, 17% of all dead or dying trees were undamaged by elephants. Second, the mean condition against height-class curve was highly correlated for both elephant damaged and non-damaged trees ($r = 0.956$, $P < 0.001$), although the latter were in significantly better condition ($P < 0.05$).

An analysis of the soil samples showed that tree stands in an advanced stage of collapse were associated with highly saline soils, while healthy stands were associated with non- or mildly saline soils (Fig. 2). A fairly narrow margin around an EC_e of 8 mmho cm^{-1} divided healthy and moribund stands. In some cases dead stands were associated with EC_e values of $50\text{--}70 \text{ mmho cm}^{-1}$, equivalent to an osmotic pressure of approximately 25 atmospheres. Such high moisture tension can be expected to inhibit water uptake in most plants, with the exception of a narrow spectrum of halophytes⁷. *Acacia xanthophloea*, a hydrophyte⁸, would not be expected to fall into this category.

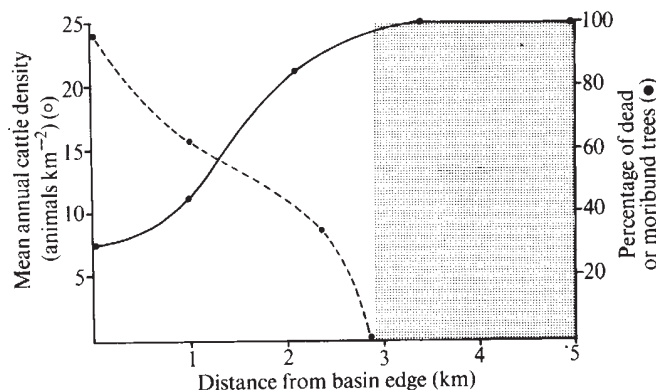


Fig. 1 The distribution of woodland mortality across a section of the Amboseli basin shows a progressive deterioration toward the centre. Cattle density, determined from monthly counts over three years, shows a reverse trend. The stock-free area (tinted) provides a useful control, demonstrating that livestock have had little influence on the major habitat changes.

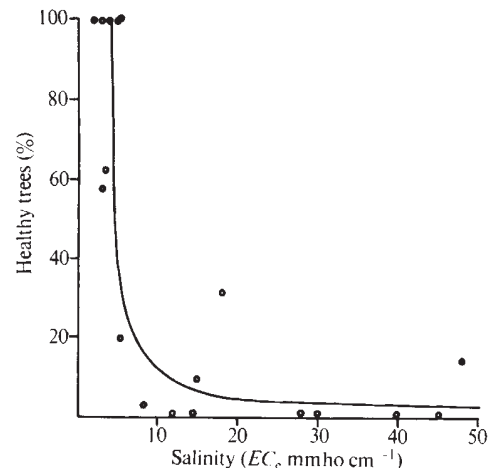


Fig. 2 For each sample plot, the percent healthy trees are plotted against the highest salinity values recorded from profiles taken through the rooting zone. In general, plots associated with an $EC_e < 7 \text{ mmho cm}^{-1}$ were in a healthy state, while those associated with an $EC_e > 8 \text{ mmho cm}^{-1}$ were in an advanced stage of collapse.

Evidence for this conjecture was derived from an examination of a dead stand of trees $> 12 \text{ m}$ in an extremely saline area. The only regeneration was composed of saplings $< 2 \text{ m}$ of which the taller individuals were dying. Analysis of the rooting system of these trees showed withering from the tips at about 40 to 60 cm depth, which from the soil profiles was found to correspond to a band of high salinity, $40\text{--}50 \text{ EC}_e$. The withering suggests tissue plasmolysis and eventual death through physiological drought on the highly saline soils. Less than 40% of these saplings showed any browse or elephant damage.

A highly significant correlation ($P < 0.001$) was also found between tree density and salinity (Fig. 3). The increasing level of salinity towards the basin centre probably explains the mortality pattern of trees noted earlier (Fig. 2), as there is a significant correlation between the two trends ($P < 0.02$).

With the available evidence strongly indicating salinity to be the primary cause of woodland mortality, it is necessary to offer some reason for the rapid and widespread salinization of Amboseli basin. The most likely explanation is an upward shift in ground water levels of some 3.5 m between 1961 and 1964, observed at Sinya Meershaummine⁹, located in the basin. Borehole records at Ol Tukai Lodge demonstrate a similar elevation to a rest height of about 3–4 m below the surface.

The high correlation of both tree condition and density with soil salinity and the associated habitat changes mentioned below suggest it is unlikely that *A. xanthophloea* death results directly from drowning except along the swamp margins. Instead, a 3 to 4 m elevation in water-table has caused the capillary fringe to introduce a high level of soluble salts into the rooting layer, causing death through physiological drought. Russell¹⁰ and More¹¹ have discussed the mechanisms of salinization due to a rising water-table or capillary fringe, and this is recognized as a common problem in irrigation engineering¹².

Lahi⁹ and Bargman¹³ have shown that increments in ground water level in Amboseli correlate with a post-1960 rainfall increase, while Campbell¹⁴ indicated a less marked rise in water levels in the 1950s. It seems possible therefore to trace the woodland mortality ultimately to changes in the rainfall regime.

The Amboseli ecosystem is apparently sensitive to changing climatic conditions. A successional series of plants is apparent within the basin and, although the disappearance of fewer trees is probably the most obvious feature of recent changes, the associated flora shows a comparable shift to halophytic species. The most conspicuous saline indicator is *Suaeda monoica* which is rapidly spreading across the basin as the woodlands decline. *Suaeda* cover was found to be inversely correlated ($r = -0.76$, $P < 0.001$) with *A. xanthophloea* across

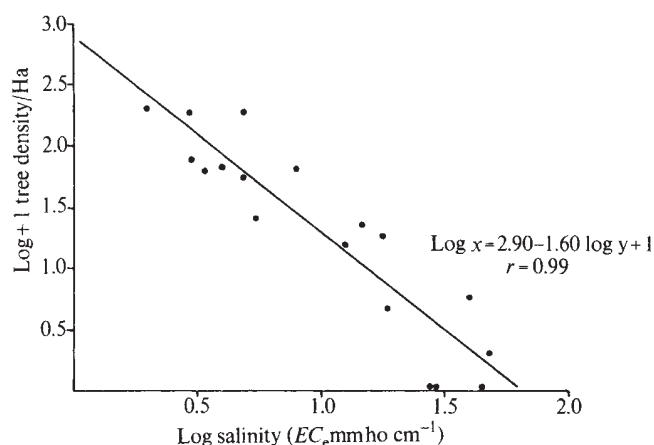


Fig. 3 Tree density for each plot was determined from aerial photomosaics, salinity as in Fig. 2. A sharp inflexion point at about 7–8 EC_e divides sparse from dense stands when plotted on a normal scale, but transformation to a log scale above gives a straight line correlation.

a section of receding woodland, the spread of *Suaeda* showing up clearly on the 1950 to 1967 aerial survey. Any major shift in water-table is likely to alter the salinity profile and affect successional series. A depressed rainfall period will result in a lowering of water levels, downward leaching of soluble salt and a shift to a non-halophytic vegetation, the converse with higher rainfall regimes.

On this basis it might be expected that a halophytic community dominated the basin during the last century when rainfall and lake levels were apparently higher¹⁵. Two lines of evidence support this hypothesis. First the Maasai record that during the period when the Nyanjusi I age-set were warriors the woodlands were almost non-existent and the swamps were far more extensive than at present. Jacobs¹⁶ age-set chronology shows that this occupied the period between 1851 and 1871. According to the Maasai, two small groves of woodland then existed, separated by *Empaash* (Maasai, a corridor). With the woodlands now largely dead or moribund, *Empaash* divides two of the few remaining healthy groves. Secondly, descriptions of Amboseli given by early explorers make no mention of extensive woodlands. Thomson¹⁷, who passed through in 1883, mentions a few "straggly trees", but no extensive woodland, while Volkens¹⁸ described the area as dominated by *Suaeda monoica* as did Wickenburg¹⁹. A number of other references suggest that the area was sparsely wooded but covered by more extensive swamps. The now seasonal Lake Amboseli was apparently permanently inundated until the 1920s²⁰. Available photographs show a relatively young woodland in the 1930s, which, coupled with the preceding evidence, suggests that the major spread of woodlands did not begin until the end of the last century or, more possibly, early this century.

The change from a hydrophytic, dense woodland to a halophytic community has resulted in a sparse xeromorphic habitat, typical of more arid conditions. This fostered the view that the area was becoming more arid due to livestock overgrazing. It has also accounted for a pronounced shift in large mammal composition for which substantial information is now accumulating.

The most obvious change has been a decline in woodland-associated species such as lesser kudu, baboon, vervet-monkey, leopard and impala. Elephants appear to play a catalytic role in the present habitat changes rather than their primary role in other areas³. The incidence of elephant damage was found to be inversely correlated with woodland density ($r=0.931$, $P<0.001$). This relationship effectively accelerates the decline already under way as a result of salinization. It also results in the highest incidence of elephant damage being associated with the sparser and more moribund stands of trees,

an association that has been readily interpreted to support the hypothesis that elephants are the primary agent of destruction.

The correspondence of the large oscillations noted over the last century in a number of East African lakes such as Victoria, Nyasa, Rudolf, and Naivasha, has been attributed to climatic changes^{15,21,22}. The major fluctuations in the Amboseli ground-water and swamp levels follow a similar periodicity, with high levels pre-1890 and post-1960 and an intervening low. In Amboseli these changes have also had a pronounced influence on the entire ecosystem, demonstrating the need for a long-term perspective of the contemporary habitat changes. It is important to consider the effect of climatic shifts on other East African habitat changes, particularly closed drainage systems such as Manyara, Naivasha, Nakuru and Ngorongoro, and possibly on changes in elephant range and activity.

A long term perspective of contemporary habitat changes is especially relevant to the future management of Amboseli. Over the next decade or so, it is envisaged that investments in the basin will exceed K£1.5 million on the assumption of deriving annual tourist revenues ultimately reaching K£1.5 to 2 million²³. In view of the recent hydrologic changes, it is important to consider the probability of investment loss through flooding, a further rise of the magnitude observed over the last decade would inundate most of the present lodge installations. It is imperative to plan developments and devise management strategies with some idea of possible future changes, because it is not economically feasible to control either the direction or magnitude of water-table shifts. A study of Holocene to present day lake and swamp level fluctuations of the type recently conducted by Butzer²¹ and Butzer *et al.*²² would help to elucidate the fluctuations and recurrence intervals that can be expected. An abundance of fish and mammalian fossils through a range of exposed strata in the basin, and a series of possible strand lines, offer ideal material for such a study.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Soft X-ray Pulsations from PSR 0833-45

THE radio pulsar PSR 0833-45 has the second fastest period of the presently known pulsars and was the first pulsar to be associated with a supernova remnant¹. The SNR is the site of the nonthermal radio source² Vela X and has also been observed to be a region of soft X-ray emission³. We have recently observed the X-ray structure of the Vela and Puppis SNRs in the energy range 0.1–1.5 keV with a scanning focusing collector that provided substantially better angular resolution than did the previous soft X-ray measurements of this region³. The instrument was an improved version of the device described by Gorenstein *et al.*⁴ and was flown aboard an Aerobee 170 sounding rocket fired from the White Sands Missile Range on April 1, 1972. We find the X-ray source in the Vela SNR to consist of a continuous extended region with structure plus a concentration of X-ray emission which is consistent with a point source at the position of PSR 0833-45. Here we report only results pertaining to PSR 0833-45.

The field of view of the instrument ($1.8^\circ \times 12^\circ$) was divided into several image elements by a thin window position sensitive proportional counter in the focal plane. In the focusing direction, the response of each element was essentially rectangular, as determined by the 0.3° counter-cell width, because the mirror resolution was finer than this ($\sim 0.1^\circ$). In the other direction, the mirror plates behaved as a slit collimator with a 6° FWHM response. Data from the various image elements have been superimposed as prescribed by the aspect data.

In Fig. 1 we show the counting rate for the energy range 0.5–1.5 keV as a function of angle during a scan along a great circle that was approximately parallel to the galactic equator but displaced by several degrees. Several features are present in the 0.5–1.5 keV data. The Puppis SNR (Pup A), which is not related to the Vela SNR, is a rather bright X-ray source with a finite angular extent that is larger than our resolution. The X-ray source in the Vela SNR consists of an X-ray nebula that is not resolvable into point sources plus a peaking of the intensity at the position of PSR 0833-45 that to within our angular resolution is consistent with a point source. The Vela SNR radio source appears in some catalogues of radio sources as three sources, Vela X, Y, Z, and in other catalogues simply as Vela X. The radio features of Vela X, Y, Z do not necessarily have a one to one correspondence with features of the X-ray source which we will call the Vela X-ray Nebula. The position of PSR 0833-45 is about two degrees removed from centre of the Vela X-ray Nebula.

For the 0.5–1.5 keV energy range the intensity of the feature at PSR 0833-45 above background of the nebula is equal to $6.2 \pm 2.6\%$ of the intensity of the entire Vela X-ray Nebula. This was determined from Fig. 1 as follows: the strength above background (dashed line) of the nebula was calculated assuming a $\sim 6^\circ$ region centred at a relative azimuth of $\sim 4^\circ$. The two bins at the PSR 0833-45 arrow were reduced by a local nebula contribution (averaged over azimuth $\sim 3.5^\circ$ to 6.5°) to give an estimated strength of the pulsar. The quoted error is the statistical component only; there is a systematic

uncertainty in determining background from the nebula in the vicinity of PSR 0833-45.

Our value of $\sim 4 \times 10^{-9}$ erg cm⁻² s⁻¹ for the integrated intensity (0.5–1.5 keV) of the Vela X-ray Nebula is in agreement with the report of Seward *et al.*³. For the energy range 0.1–0.3 keV there is no indication of an increase in intensity at PSR 0833-45 with the 3σ upper limit (4.5% of the intensity of the Vela X-ray Nebula). Thus the X-ray spectrum from the direction of PSR 0833-45 seems to be harder than the spectrum of the Vela X-ray Nebula. This conclusion is consistent with observations from Uhuru above 2 keV where there does seem to be localized X-ray emission about the position of PSR 0833-45; the Vela X-ray Nebula (except possibly for isolated fragments) is not detectable in the Uhuru energy range⁵.

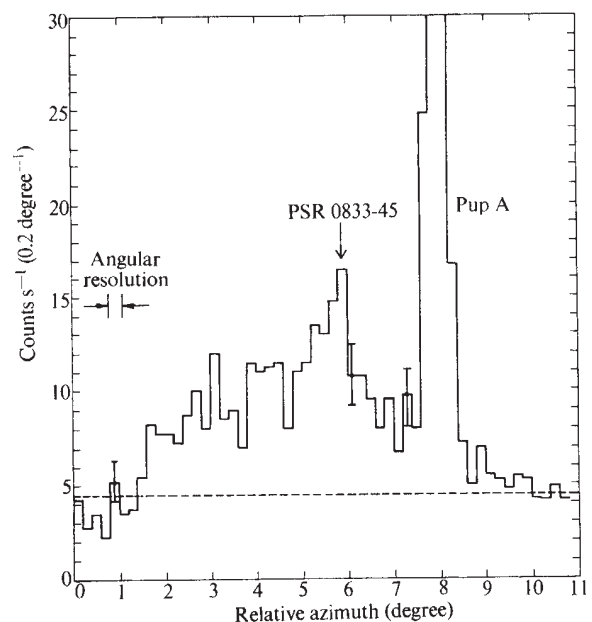


Fig. 1 Angular structure of 0.5–1.5 keV X-rays during scan along galactic equator. The dashed line is the background level outside of the Vela-Puppis region. The bright X-ray source is coincident with the radio source Pup A. The region with a 6° extent is the Vela X-ray Nebula. The position of PSR 0833-45 is indicated.

We have investigated the temporal behaviour of X-rays from the direction of PSR 0833-45 to search for possible periodic pulsations. The data from the PSR 0833-45 vicinity (a region $0.3^\circ \times 12^\circ$) include 6 s of observation and a total of only some 200 counts, more than 4/5 of which could be accounted for by the sum of the contributions from the nebula and background. The period of the radio pulsar PSR 0833-45 at the time of the X-ray observation was 0.08922 s (Reichley and Downs, private communication) at 2,388 MHz. The result of folding the X-ray data about this period is shown in Fig. 2. One bin contains substantially more counts than the mean. On the basis of Poisson counting statistics, the probability that the effect we observe at the period of the radio pulsar is a chance

occurrence is only $\sim 2 \times 10^{-3}$. We have also analysed our data for unsuspected instrumental periodicities over a wide range of periods and in doing so have verified that the occurrence rate of large deviations from the mean is no greater than expected.

The phase of the radio pulse at the time of the X-ray observation (derived from radio observations of Reichley and Downs made only 30 min before the rocket flight) has been corrected for an estimated ~ 0.050 s propagation delay in the interstellar medium and is shown in Fig. 2 in comparison to the X-ray. There is a difference in arrival times of about 0.029 s between the purported X-ray pulse and the radio pulse. Assuming that there is no gross time synchronization error in the X-ray data records which we have failed to uncover, there are two possible interpretations for the timing discrepancy. First, the X-ray effect is spurious. We think this is unlikely on the grounds that the probability of observing such an effect by chance is rather small. Also there is the evidence for a point-like X-ray feature at the position of PSR 0833-45 which is distinguished from the Vela X-ray Nebula by a harder spectrum and a location removed from the centre of the nebula. The other interpretation is that the time difference has a physical basis. The difference could be due to the existence of an interpulse from PSR 0833-45. If so the main pulse would be strong at radio frequencies and weak in X-ray emission while the reverse would be true of the interpulse. This situation does occur to an extent in the case of NP 0531, the pulsar in the Crab Nebula, where the relative intensity of the main to interpulse varies from one region of the electromagnetic spectrum to another⁶.

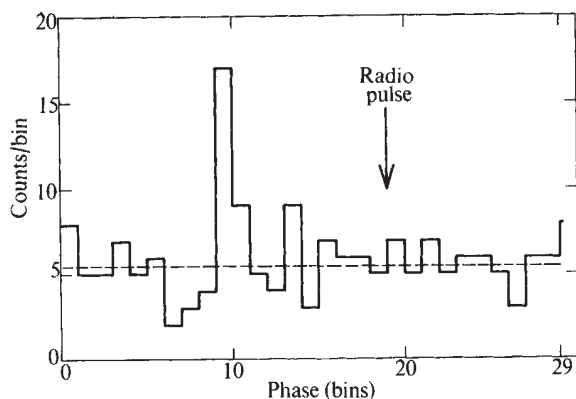


Fig. 2 Distribution in phase of counts detected from vicinity of PSR 0833-45 for an assumed period of 0.08922 s. The phase of the radio pulse is also shown. The dashed line is the average per bin excluding bin 10.

Relative to the Vela X-ray Nebula, the pulsed fraction inferred from Fig. 2 is about 2% in the energy range 0.5–1.5 keV. This is not in conflict with the previous upper limit of 1% reported by Seward *et al.*³ at slightly lower energies because the pulsar is favoured relative to the nebula at higher energies. Further, the improved angular resolution of the present result entirely eliminates any contribution from Pup A. Thus the pulsed fraction of a point source at the position of PSR 0833-45 is about 1/3 but is subject to the systematic uncertainty described above. For the energy range 0.5–1.5 keV the time averaged intensity of the pulsed component is $7.4 \pm 2.8 \times 10^{-11}$ erg cm⁻² s⁻¹. The intensity within the pulse is a factor of 30 ($2.2 \pm 0.8 \times 10^{-9}$ erg cm⁻² s⁻¹).

If the X-ray pulsations are real, then in spite of the great difference in age and intrinsic diameter between the two nebulae there are several points of similarity between the relative intensities of PSR 0833-45 to the Vela X-ray Nebula

and NP 0531 to the Crab Nebula. First, PSR 0833-45 has a harder spectrum than the Vela X-ray Nebula as does NP 0531 compared to the Crab⁶. In particular there is some evidence for emission from PSR 0833-45 above 23 keV (ref. 7). At these energies the Vela X-ray Nebula should no longer be detectable. Second, in both cases the X-ray luminosity of the nebula is about an order of magnitude smaller than the kinetic energy per second lost by the slowing down of a rapidly rotating neutron star whose rotation rate is equal to the pulsar period. Third, the time average of the beamed intensity of the pulsar received at the Earth is less than 10% of the intensity of the nebula in the energy range 0.5–1.5 keV but is a larger fraction at higher energies. One or more of these similarities may be coincidental. There is little doubt that essentially the entire X-ray luminosity of the Crab Nebula is due to synchrotron radiation from electrons accelerated to relativistic energies by NP 0531. Thus by analogy, one might ascribe the luminosity of the Vela X-ray Nebula to PSR 0833-45. But Tucker⁸ has pointed out that under the conditions found in the Vela SNR at least part of the luminosity of the Vela X-ray Nebula could be thermally produced by the propagation of a blast wave in the interstellar medium. The Crab Nebula has not yet evolved to a stage where this process would be a factor in its X-ray emission.

The detection of pulsations, in addition to localized emission about PSR 0833-45, strengthens the case that the pulsar is a point source of X-rays. This should remove any residual doubt about its intimate connexion with the Vela SNR. The presence of pulsations also provides a basis for discriminating between various models of conditions in the vicinity of a rapidly rotating neutron star. The failure of intensive searches⁹ to observe an optical counterpart for PSR 0833-45 implies that at visible wavelengths the Crab pulsar is at least 1,000 times brighter than the Vela pulsar, while at X-ray wavelengths it is only about twice as bright. This result favours pulsar models such as the one of Sturrock¹⁰ in which a neutron star rotating three times more slowly than NP 0531 is still an active source of X-rays but emits no visible light. In this model the very low ratio of visible light to X-rays of PSR 0833-45 as compared to NP 0531 is a natural consequence of its longer rotational period.

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A Limit on the Redshift due to Interaction with Electromagnetic Radiation

In a recent letter¹ Pecker *et al.* discussed the possibility that a part of the "cosmological" redshift could result from the interaction of photons with an interstellar radiation field. Although this suggestion is old^{2,3}, it has recently regained prominence with the discovery of the 3 K blackbody radiation. Pecker *et al.* discussed a formula for the energy loss of the propagating photon, v_1 ,

$$-\delta v_1/v_1 = \Delta(v_2)n_2\delta l \quad (1)$$

where $\Delta(v_2)$ is the coupling constant between the propagating photon and the radiation field of frequency v_2 , n_2 is the number density (cm^{-3}) of the photons at v_2 , and δl is the length of the interaction path, in cm. Assuming that $\Delta(v_2)$ is independent of v_2 , they suggested an experiment to determine an upper limit for its value. The proposed experiment, measuring by the Mössbauer effect the energy loss of gamma rays traversing a laser beam, is very difficult to perform. But another Mössbauer experiment was performed a number of years ago to test just this question. In that experiment⁴, gamma rays were propagated through a microwave cavity filled with photons of 9,234 MHz ($\lambda \approx 3$ cm) radiation; the fractional energy shift of the gamma rays due to the microwave field was found to be $0.4 \pm 6.0 \times 10^{-16}$, corresponding to a value of $\Delta(v_2) < 10^{-33} \text{ cm}^{-1}$. This limit is far smaller than the value $\Delta(v_2) \approx 10^{-30} \text{ cm}^{-1}$ proposed in ref. 1 from the analysis of solar redshift data.

We note that the 3 cm wavelength of the microwave radiation used in the experiment of ref. 4 lies near the peak ($\lambda_{\text{max}} \approx 0.1$ cm) of 3 K blackbody radiation. Thus the microwave experiment establishes a useful and reliable upper limit for $\Delta(v_2)$, provided the Doppler form of equation 1 is valid. This limit is far too small to account for the observed cosmological redshift which requires¹ $\Delta(v_2) \approx 10^{-30} \text{ cm}^{-1}$.

J. A. Giordmaine brought this problem to our attention; we thank J. R. Klauder for discussions.

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Consequences of a Universal Cosmic-ray Theory for γ -ray Astronomy

THE problem of the origin of cosmic rays is well known; but in spite of considerable advances in knowledge concerning energetic extra-terrestrial objects in recent years, no satisfactory solution has appeared. It is not clear, even, whether the important sources are within the Galaxy or outside it^{1,2}.

Here we consider the possibility of reviving a theory due to Hillas³ in which the observed shape of the primary spectrum up to about 3×10^{19} eV is explained in terms of an evolving sources model with constant spectral index at production, and interactions with the microwave background radiation. Such a model can explain the sharp change of slope at 3×10^{15} eV inferred from measurements of the sizes of extensive air

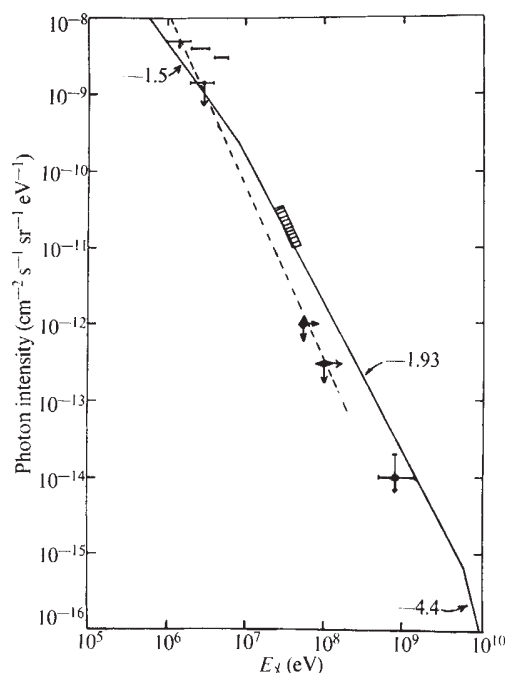


Fig. 1 The expected γ -ray spectrum (solid line). $\blacklozenge$, Ref. 9 (OSO-3); $\blacklozenge$, ref. 8 (COSMOS-208); $\blacksquare$, ref. 8 (PROTON-2); --- , ref. 10 (COSMOS-163); --- , ref. 11 (ERS-18); --- , ref. 10 ($E^{-2.4}$ fit to experimental data); hatched , ref. 12 (balloon experiment).

showers, as well as the high degree of isotropy which is maintained up to at least 10^{19} eV (ref. 4).

The objections so far advanced to theories of this type seem to rely on assumptions concerning unknown astrophysical quantities. Thus the problem of a γ -ray excess due to pionization on intergalactic matter⁵ can be removed by assuming a low enough density for the intergalactic medium. An X-ray excess is clearly expected if the proton/electron ratio is equal to its local value, but in the absence of evidence to the contrary the possibility that this ratio may be large enough in metagalactic sources to remove the discrepancy cannot be ruled out.

A more fundamental objection would arise if it could be shown that the energy lost in forming the observed primary spectrum above 3×10^{15} eV (which appears initially in the form of electron pairs in this type of theory) will result in a γ -ray flux inconsistent with measurements of the diffuse γ -background. The mechanisms involved are inverse-Compton effect on the microwave background and pair-production on the starlight background, the latter occurring within the Hubble radius for γ -ray energies above about 10^{11} eV for reasonable estimates of the starlight background (see, for example, ref. 6). It is assumed that the starlight intensity does not increase with z —such changes as might be expected do not affect the results greatly in the energy range of interest to us (10^6 to 10^9 eV).

We have made calculations for a model in which the energetic cosmic rays are protons, with an energy spectrum on production of the form $G(E_p, z)dz = BE_p^{-\gamma} f(z)dz$, where B is a constant, z is redshift and $\gamma = +2.6$. The source efficiency $f(z)$ is taken as

$$f(z) = H_0^{-1} \frac{(1+z)^{\beta}}{(1+z)^2 (1+2q_0z)^{\frac{1}{2}}} \quad \text{for } z < z_m$$

$$f(z) = 0 \quad \text{for } z > z_m$$

corresponding to the form used by Longair⁷ in treating the evolution of powerful radio sources. In the calculations we took $q_0 = \frac{1}{2}$, although the results are insensitive to this parameter.

The proton spectrum expected at the present epoch after the generated protons have interacted with the microwave background has been calculated for various values of z_m and β . Comparison with the measured spectrum yields best-fit values $z_m = 14.3$ and $\beta = 4.3$. (The problem of predicted spectrum falling more rapidly above 10^{19} eV than observed is not considered here.) With these values the rather rapid change of slope of the the primary spectrum near 3×10^{15} eV is well represented.

The values for z_m and β can be compared with those derived by Longair from source counts ($2.3 < z_m < 4$ and $\beta = 5.7$ for density evolution, 3.3 for luminosity evolution). Our value of $z_m = 14.3$ is clearly inconsistent with the radio-source value but this is not catastrophic because cosmic-ray sources and radio sources need not evolve in the same time scale.

We show the expected γ -ray spectrum in Fig. 1. (The sharp changes of slope are consequences of approximations in the calculations.) Most of the energy is contained in the region where the slope is -1.93 so that the energy content per unit logarithmic interval is almost constant and the result is not very sensitive to the adopted parameters of the starlight spectrum.

Also shown are the experimental measurements of the diffuse γ -background from satellite and balloon experiments. The intensities measured by OSO-3, PROTON-2 and COSMOS-208 are clearly below the prediction of the present work. The measurements reported by Meyer-Hasselwander *et al.*¹² agree with our prediction.

On balance the observed intensities are too low and we conclude that what appeared to be a very attractive model is untenable. This conclusion will also apply to a "hybrid" model of the type suggested by Beresinsky and Zatsepin¹³ in which galactic origin below 10^{15} eV is combined with a Hillas-type metagalactic spectrum above 10^{15} eV.

Note added in proof. Of course, if the "high" intensities of ref. 12 are confirmed by later work, then the conclusion will be reversed.

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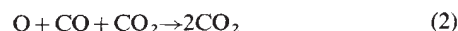
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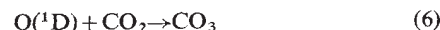
Stability of CO₂ in the Martian Atmosphere and under Radiolysis

THE atmosphere of Mars appears to be predominantly undissociated CO₂ (refs. 1–3). It was possible by assuming very rapid transport downwards to explain the composition of the upper atmosphere, but a problem remained at lower altitudes.

More recent determinations^{4,5} of the rate of O–CO recombination have, however, given a rate constant two orders of magnitude lower than that used in the calculations for 200 K, the Martian surface temperature. This creates a more severe problem. There are less CO and O₂ than can be accounted for by a mechanism of photolysis and recombination.



An earlier suggestion² that further reactions involving CO₃ could explain the results is not correct^{1,3}.

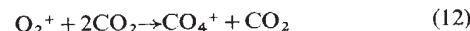
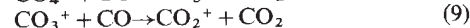
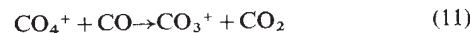


Furthermore, reaction (6) without a third-body or as a radiative process seems highly unlikely, and recent experiments yield no evidence for its existence⁶. It has also been shown that a pure CO₂ atmosphere is photochemically unstable under conditions where O (¹D) would be produced⁷. CO₂ does, however, show an apparent stability under reactor radiolysis⁸; isotope-exchange experiments have confirmed that both dissociation and recombination occur. Similar behaviour should be expected on Mars and it is pertinent to look for a common explanation.

The principal difference between photolysis and radiolysis lies in the production of ions under high-energy irradiation. The production of fragments and metastables would be expected to be somewhat similar in both cases. An ionic mechanism may therefore explain the recombination in CO₂. Although it has been shown^{9,10} that earlier explanations of radiolytic stability based on negative-ion chemistry were unsatisfactory, positive-ion processes cannot be ruled out at the present time. It is known that, in the absence of CO₂, CO is rapidly oxidized by O₂ under irradiation, and the following mechanism has been proposed¹¹.



In CO₂ the terminal positive ion under radiolysis is probably CO₄¹⁰ and the existence of the same species in the Martian atmosphere¹² has been suggested. The following modified positive-ion mechanism could oxidize CO in the presence of CO₂.



C₂O₄⁺, clustered CO₂⁺, probably also charge transfers to O₂ (ref. 13).

At altitudes below 70 km on Mars, the positive-ion density is 10^3 cm^{-3} (ref. 12) and the rate of oxidation of CO by positive ions will be $\sim 10^3 (k_{11} + k_9) \text{ s}^{-1}$. The rate of production of CO by photolysis¹ $\sim 5 \times 10^5 \text{ cm}^3 \text{ s}^{-1}$ to give a steady-state density of $500/(k_{11} + k_9) \text{ molecule cm}^{-3}$. The observed CO/CO₂ ratio is 0.1% and the CO₂ density falls from $2.25 \times 10^{17} \text{ molecule cm}^{-3}$ at the surface to 2.25×10^{15} at an altitude of 60 km, giving a CO density ranging from 2.25×10^{14} to 2.25×10^{12} . If any effects arising from transport are neglected, values of k_{11} and k_9 of the order of $10^{-10} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$ are needed to explain the observations. The radiolytic problem¹⁰ calls for rates $> 10^{-12}$.

Further experimental measurements are needed to assess this mechanism. If k_{11} and k_{12} are found to be too small, then it would appear that minor constituents are responsible for promoting the oxidation of CO. Besides the well-established effect of H_2O at high temperatures, catalytic effects on CO oxidation arising from iron carbonyl¹⁴ and perhaps also CH_4 (ref. 15) have recently been noted. Impurities also enhance the apparent rate of oxygen atom recombination with CO^5 .

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Earth-Moon Mass Ratio from Mariner 9 Radio Tracking Data

THE navigation of the Mariner 9 spacecraft from Earth to Mars was performed using phase-coherent range and doppler tracking data recorded by the Jet Propulsion Laboratory (JPL) Deep Space Network. These data also determine the Earth-Moon mass ratio, which involves the following physics: as the Earth revolves about the centre of mass of the Earth-Moon system, a sinusoidal curve is impressed on the range and doppler tracking data with a frequency equal to the sidereal mean motion of the Moon. This signature is shown in Fig. 1, where a perturbation of 0.0003 was made in the mass ratio

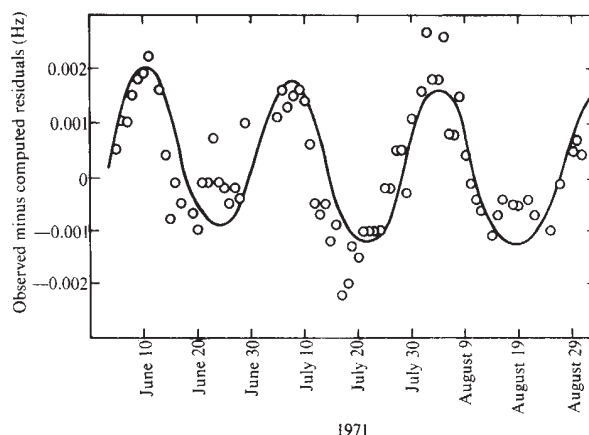


Fig. 1 The effect of Earth-Moon mass ratio on doppler residuals.

(μ^{-1} = mass of Earth over mass of Moon). This sinusoidal variation in the tracking data can be eliminated by finding a value for μ^{-1} that properly represents the amplitude of the barycentre motion of the Earth. The procedure is direct and for all practical purposes is completely uncoupled from other parameters used in reducing the tracking data.

The mass ratio was determined from range and doppler data obtained over a period of 15 weeks (June 5 to September 15, 1971). The data coverage is shown in Table 1. We also show the statistics from the best determination. The data reduction was performed using the JPL Double Precision Orbit Determination Program¹, which uses a Cowell integrated trajectory and a batch least squares filter. In weighting the range data, we have taken extreme care to assure optimal data utilization without conflicting with the doppler data. The Mariner Mars 1969 results showed that such conflicts can cause significant perturbations in the estimated parameters.

The Deep Space Network has three types of ranging systems: Mu, Tau, and Mark 1A. The Mu and Tau systems are capable of planetary distances, whereas the Mark 1A ranging system is limited to an effective one-way range of approximately 10^7 km. In weighting the range data the following factors were taken into consideration. First, assuming no external errors, the Mu and Tau systems are accurate to about 20 m. This includes system noise and transponder and ground equipment calibration errors. The Mark 1A ranging system is accurate to about 30 m. Second, because the radio signals travel through the ionosphere of the Earth and the interplanetary space plasma, there is a change in the radio signal path length. The group wave path length is increased, while the phase wave path length is decreased, corrupting both range and doppler measurements made from the radio signal. The charged particles of the

Table 1 Tracking Data Statistics

Tracking station	Data type*	Number of points†	Data interval, 1971 (UTC)	Mean residual‡	Root-mean-square residual‡
Goldstone-Echo, Calif.	Doppler	1,069	6/5 08:37 to 9/14 04:59	0.000005 Hz	0.00144 Hz
	Range-Mark 1A	248	6/5 08:42 to 6/30 09:14	-17.73 RU	20.94 RU
	Range-Mu	31	7/12 05:40 to 9/6 02:49	-19.12 ns	134.55 ns
Goldstone-Mars, Calif.	Doppler	193	6/5 13:01 to 8/31 09:36	0.000002 Hz	0.00132 Hz
	Range-Tau	109	6/5 12:43 to 8/27 06:20	-33.67 ns	116.22 ns
Woomera, Australia	Doppler	1,638	6/5 01:15 to 9/12 17:56	-0.000012 Hz	0.00139 Hz
	Range-Mark 1A	594	6/5 01:09 to 7/13 17:40	5.28 RU	19.21 RU
Johannesburg, South Africa	Doppler	1,336	6/5 02:54 to 9/14 22:57	-0.000117 Hz	0.00140 Hz
	Range-Mark 1A	776	6/5 03:00 to 7/18 04:31	4.38 RU	20.60 RU
Cebreros, Spain	Doppler	387	6/26 00:43 to 9/12 00:36	0.000023 Hz	0.00188 Hz
	Range-Mark 1A	27	7/5 02:40 to 7/15 02:50	1.97 RU	21.04 RU

* Mark 1A = near-Earth ranging system; Tau and Mu = ranging systems using different ground hardware.

† Sample rate for doppler and range was 20 min.

‡ 1 Hz = 65 mm/s; 1 RU (range unit) $\approx$ 1 m; 1 ns $\approx$ 0.15 m.

Earth's ionosphere could account for as much as a 15 m error in range; the charged particles in the interplanetary medium (space plasma) could account for as much as a 22 m error in range (J. F. Jordan *et al.*, paper presented at AAS/AISS Astrodynamics Conference, August 1970). Third, another possible cause of range error is the Z component of station location. This component is parallel to the Earth's spin axis. The computed range value is sensitive to incorrect Z values when the probe declination becomes large in absolute value. An equation relating the two is³

$$\Delta\rho = \Delta Z \sin \delta$$

where ρ is the range datum and δ is the geocentric declination of the spacecraft.

Because previous space mission data did not yield significant information on the Z component of station locations, JPL analysts used the Z values obtained by the Smithsonian Astrophysical Observatory (SAO) in 1969. The change in the Z component was as much as 56 m. Assuming that the Z values from SAO may be in error by 30 m and the maximum absolute value in geocentric declination for Mariner Mars 1971 is 29.15°, the above equation would yield a range error of 14.6 m. The doppler data are insensitive to this Z -component error. Even though the Z component of a station location is not too well determined, the distances from the spin axis and the longitude are known to better than 3 and 5 m, respectively.

A number of solutions with different combinations of weights for each data type and different sets of estimated parameters were examined. The standard set of estimated parameters includes the probe position and velocity (6), solar pressure (3), attitude control leaks (3), station locations (15), and the Earth-Moon ratio (1). An *a priori* statistic of 0.0166 was applied to the mass ratio parameter. These solutions and their identifications are given as follows:

Case 1, Doppler only (doppler weight=0.015 Hz) with standard estimated parameter set. Case 2, Range only (range weight=100 m) with standard estimated parameter set. Case 3, Doppler and range (doppler weight=0.015 Hz, range weight=100 m) with standard estimated parameter set. Case 4, Doppler and range (doppler weight=0.015 Hz, range weight=100 m) with standard estimated parameter set plus Mars and Earth-Moon barycentre ephemeris parameters. Case 5, Doppler and range (doppler weight=0.015 Hz, range weight=50 m) with the estimated parameter set as in Case 4.

Solutions	μ^{-1}
Case 1	81.30068
Case 2	81.30067
Case 3	81.30067
Case 4	81.30067
Case 5	81.30068

All solutions yielded nearly the same mass ratio. Cases 1 and 2 give remarkable agreement on a mass ratio between the two data types. With such good agreement, the relative weight of the two data types becomes less significant. Cases 3 and 4 show that the lunar ephemeris error is probably too small to have an effect on the mass ratio estimate. Possible error sources are the periodic variations in the interplanetary medium. W. G. Melbourne has shown (12th Plenary Meeting of the Committee on Space Research, Prague, 1969) that a 28 day sinusoidal variation of solar flux of 0.1% could produce an error of about 0.001 in the mass ratio, but that it is not likely. Also, the agreement of mass ratios computed from the data gathered from several interplanetary spacecraft does not indicate this sort of systematic error unless the phase of the flux variation is the same for each mission, which does not seem likely.

The results from the Mariner Mars 1971 data are given in Table 2 together with previous results obtained from Pioneers 8 and 9 and Mariners 2, 4, 5, 6 and 7. Values computed for Pioneers 8 and 9 and Mariner 2 were obtained from solutions using only doppler data. It is interesting to note that the

Table 2 Estimates of the Earth-Moon Mass Ratio, μ^{-1}

Spacecraft	μ^{-1}	$\mu^{-1} - \bar{\mu}^{-1}$	Reference
Pioneer 8	81.3004 ± 0.0001	-0.0003	5
Pioneer 9	81.3008 ± 0.0001	0.0001	5
Mariner 2 (Venus)	81.3001 ± 0.0013	-0.0006	6
Mariner 4 (Mars)	81.3015 ± 0.0017	0.0008	7
Mariner 5 (Venus)	81.3013 ± 0.0002	0.0006	5
Mariner 6 (Mars)	81.3005 ± 0.0002	-0.0002	5
Mariner 7 (Mars)	81.3005 ± 0.0002	-0.0002	5
Mariner 9 (Mars)	81.3007 ± 0.0001	0.0000	

$\bar{\mu}^{-1}$ = arithmetic mean.

Mariner 9 value and the mean of all spacecraft determined values of μ^{-1} are 81.3007. The deviations from the arithmetic mean of the Mariner and Pioneer values are tabulated in Table 1. Further, the mass ratio computed from the last five interplanetary spacecraft launched (Pioneers 8 and 9 and Mariners 6, 7 and 9) showed a spread of only 0.0004. The values for Pioneers 8 and 9 and Mariners 6, 7 and 9 are 81.3004, 81.3008, 81.3004, 81.3005 and 81.3007, respectively. This provides a good indication of the accuracy of μ^{-1} . The decrease in the fluctuation of the mass ratio value can be attributed to the improvement of data quality owing to changes in Deep Space Network tracking systems and the change in computer software from single precision to double precision.

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Predicting Light Flashes due to α -Particle Flux on SST Planes

THE astronauts who flew on Apollo Lunar Missions 11 to 15 reported the observation of light flashes at a rate about one per minute when they were travelling outside the geomagnetic sphere of the Earth and their eyes were dark-adapted¹. Experiments to determine the causes of the phenomenon²⁻⁶ confirm that the flashes were caused either by direct interaction between energetic heavy primary cosmic rays or neutrons and the retina, or by Čerenkov radiation produced by muons. We have already reported⁷ that the flux of stopping cosmic nuclei of medium and heavy charges at the levels at which SSTs fly (60,000 to 70,000 foot) is of the order of 10^{-4} [cm²-min-ster-(MeV/n)]⁻¹ with a medium energy of about 120 MeV/n. If every nucleus in these categories could produce a flash in the human eye, the frequency of light flashes would be 2 to 3 per hour for an eye of 2.5 cm diameter subtending a solid angle of 2π radian. But it was noticed in the Berkeley experiments^{8,9} that fast alpha particles (up to 240 MeV) did produce flashes and streaks when they stopped inside the eye or penetrated through the retina. Because the α -particle flux is much higher than that of heavy nuclei in primary cosmic rays, the possibility of observing light flashes on SST planes owing to α -particles cannot be ruled out. We have investigated data obtained from a US Air Force plane during 80 h of supersonic high altitude flight in 1970.

The plane, an RB-57F, carrying an emulsion-plastic stack, flew over Alaska during the non-flare period from June 19,

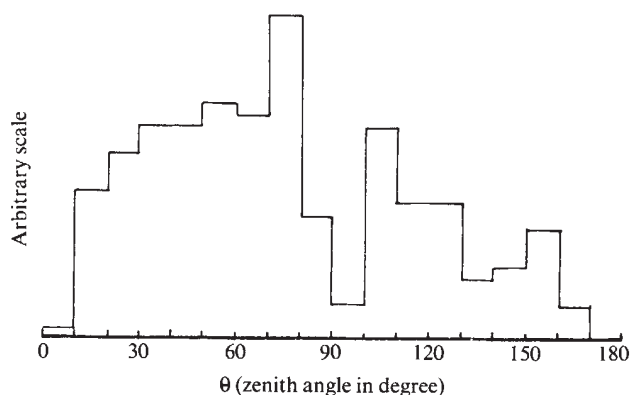


Fig. 1 Angular distribution of α -particle flux as function of zenith angle (θ).

1970 to August 10, 1970. The maximum geomagnetic cut-off rigidity was 0.1 GV. The flight time of the airplane at SST altitudes amounted to a little more than 53 h. The details of the flights have been described elsewhere¹⁰.

The detector stack contained 48 plates of G5 emulsions, each 10 cm $\times$ 10 cm $\times$ 600 μ m. Twenty-four plates in succession were under area-scanned for the ending tracks left by protons and α -particles. The relative abundance between the two categories was estimated by randomly selecting a number of tracks of an approximately equal length and identifying their charges by use of δ -ray counting.

Our results show that about one third of the stopping α -particles found in the stack ended within the top 6 plates. In terms of thickness of the human eye, filled with a vitreous fluid which is 98% water, we have estimated a total flux of about 0.7 α -particles $\text{mm}^{-2} \text{h}^{-1}$. If the cross-section of the eye is 500 mm^2 , the total number of α -particles stopping in the eye would be 350 h^{-1} . But most of these particles would stop in the vitreous body, only 11 h^{-1} hitting the retina if the thickness of retina is 2.5 mm. If each α -particle stopping at retina can cause one flash, we expect to observe 11 flashes h^{-1} during the high altitude part of an SST flight.

In order to examine this problem from the energy point of view, we present the energy distribution of the observed α -particle flux in Table 1.

Table 1 Energy Distribution of α -Particles Detected at SST Levels

Energy (MeV)	α -particle flux (α -particles $\text{mm}^{-2} \text{h}^{-1} \text{MeV}^{-1}$)
20	4.30×10^{-3}
40	3.75×10^{-3}
60	3.45×10^{-3}
80	3.10×10^{-3}
100	2.60×10^{-3}

α -Particles with energy equal to or greater than 62 MeV can penetrate through the eye. If we assume that these α -particles can also induce light flashes, an SST passenger should observe 75 flashes h^{-1} . This sounds alarming.

We emphasize, however, that we have encountered a number of difficulties in carrying out the analysis. First, the proton background was too high. According to our measurements the ratio between α 's and protons is about 1:12, which is of the same order as reported by McNaughtan¹¹ for an altitude of 90,000 foot. Second, the stack consisted of plastics and emulsions, which introduced inaccuracy to the energy estimation. Third, the α -particles seemed to have entered the stack from all directions (see Fig. 1). This might be due to the variable orientation of the aircraft during the flights. In view of these difficulties, our results can only be taken as a rough reference for more accurate measurements.

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Differential Raman Scattering of Right and Left Circularly Polarized Light by Asymmetric Molecules

It has been suggested that Rayleigh and Raman scattered light from optically active molecules should show polarization effects which could provide new information about their structure and configuration¹⁻³. Circular intensity differentials (CIDs) defined by²

$$\Delta = (I^R - I^L)/(I^R + I^L)$$

where I^R and I^L are the scattered intensities in right and left circularly polarized incident light, have recently been observed in the Raman spectra of α -phenylethanol and α -phenylethylamine⁴. (Raman CID was first reported by Bosnich et al.⁵, using α -phenylethylamine, but it has been suggested that the results are spurious⁴.) Observations now reported on another liquid, α -phenylethylisocyanate, could indicate that these differentials originate in bending vibrations around the asymmetric carbon atom.

We have observed a large Raman CID couplet in α -phenylethylisocyanate, consisting of a positive and a negative component associated with two weakly polarized bands at $\sim 223 \text{ cm}^{-1}$ and $\sim 313 \text{ cm}^{-1}$. The component of the light scattered at 90° and linearly polarized parallel to the scattering plane was sampled because, for reasons discussed previously⁴, only this component was free of spurious contributions in our instrument. The peaks of the lower and higher frequency bands are associated with values of $|\Delta| \sim 2 \times 10^{-3}$ and $\sim 1.7 \times 10^{-3}$, respectively. Much smaller CIDs were found in other parts of the spectrum, including one at $\sim 345 \text{ cm}^{-1}$. The (+)- and (-)-enantiomers give mirror image couplets. The CID couplets observed previously⁴ in α -phenylethanol and α -phenylethylamine are associated with Raman bands in both molecules at $\sim 315 \text{ cm}^{-1}$ and $\sim 365 \text{ cm}^{-1}$ with values of $|\Delta| \sim 1 \times 10^{-3}$ and $\sim 0.5 \times 10^{-3}$, respectively.

Comparison with the Raman spectrum of CHFCIBr^6 indicates that the two vibrations giving rise to the isocyanate CID couplets originate in a degenerate parent vibration in a

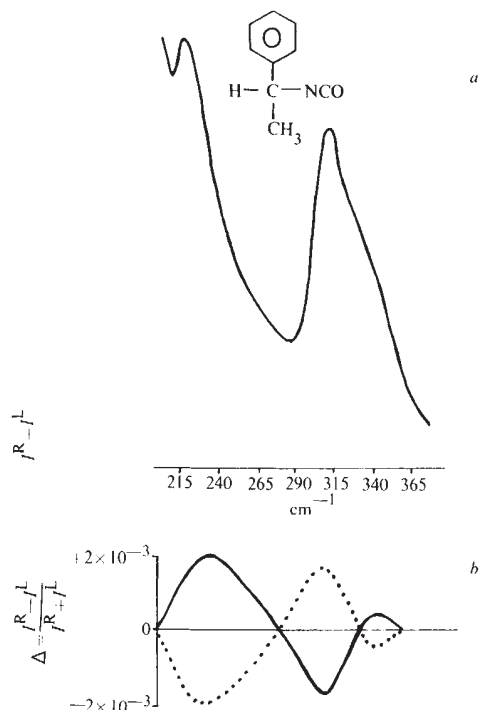


Fig. 1 Raman (a) and Raman CID (b) spectra of α -phenylethylisocyanate for scattered light linearly polarized parallel to the scattering plane. —, (–)-enantiomer; ····, (+)-enantiomer. The background was subtracted from the Raman intensities in estimating Δ . The absolute value of Δ for the band at $\sim 223 \text{ cm}^{-1}$ is uncertain since the background is still large in this region. Raman spectrum, slit width 2 cm^{-1} ; Raman CID spectrum, slit width 6 cm^{-1} .

molecule of higher symmetry (c.f. the generation of circular dichroism couplets in degenerate parent electronic transitions which are split by a lowering of symmetry). If the groups attached to the asymmetric carbon atom are regarded as atoms of equivalent mass, the vibrations around the asymmetric carbon atom in $\text{CH}(\text{CH}_3)(\text{NCO})(\text{C}_6\text{H}_5)$ should have frequencies close to those of the nine fundamentals of CHFClBr . Thus CHFClBr has two fundamentals at $\sim 225 \text{ cm}^{-1}$ and $\sim 313 \text{ cm}^{-1}$ which originate in a doubly degenerate vibration in CHX_3 (ref. 6): these frequencies correspond closely to those at which the CID couplet occurs. Unfortunately this argument cannot be extended directly to $\text{CH}(\text{CH}_3)(\text{OH})(\text{C}_6\text{H}_5)$ and $\text{CH}(\text{CH}_3)(\text{NH}_2)(\text{C}_6\text{H}_5)$ because only one of the frequencies at which the couplets occur coincides with the appropriate frequencies in the "equivalent" molecule CHF_2Br ($\sim 240 \text{ cm}^{-1}$ and $\sim 323 \text{ cm}^{-1}$ (ref. 7)). Actually the fundamental at $\sim 240 \text{ cm}^{-1}$ was not observed in the Raman⁸ spectrum of CHF_2Br , and no band was observed in this region in the Raman spectrum of $\text{CH}(\text{CH}_3)(\text{OH})(\text{C}_6\text{H}_5)$ or $\text{CH}(\text{CH}_3)(\text{NH}_2)(\text{C}_6\text{H}_5)$.

These results indicate that a method exists for directly probing the stereochemistry of asymmetric carbon atoms, which is not possible with electronic optical rotatory dispersion and circular dichroism as chromophoric electrons are not delocalized over asymmetric carbon atoms. The larger CIDs in the isocyanate indicate that the effect is a function of the differences in the masses of the four atoms or groups attached to the asymmetric carbon atom or of the corresponding mass-weighted force constants. If so, Raman CID could provide a better criterion than rotatory power (which depends on subtle and sometimes unpredictable electronic effects) for correlating absolute configurations.

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Lift and Drag Measurements on a Hydrofoil in Dilute Polyox Solutions

THE use of hydrofoils for a wide variety of purposes such as propeller blades on boats, as sailboat keels, ship rudders, submarine and torpedo fins, lifting surfaces of hydrofoil boats, and shroud ring stabilizers for missiles, has prompted efforts to increase the lift-to-drag ratio by various means. The favourable drag reducing effect of polymers, particularly for flows in pipes¹, suggests that they might be of value in increasing this ratio.

The experimental apparatus, described in detail elsewhere², consisted of a circulating water tunnel with a test section 10 cm by 20 cm; lift, drag, and pressure measuring devices; and a rheometer similar to that used by Hoyt³. The hydrofoil (NACA-0024), made of polished aluminium, was 10 cm wide, 10 cm long, and had a maximum thickness of 2.44 cm. The Reynolds number, based on the maximum thickness, ranged from 5×10^4 to 3×10^5 . The polymer used in these studies was a high molecular weight additive, 'Polyox WSR-301'.

Appropriate amounts of polymer powder were sprinkled, over a few minutes, into the test section as the pump ran at its lowest speed, thus wetting and separating the particles. The friction-reducing effectiveness λ of the solution, determined with the turbulent-flow pipe rheometer and calculated from

$$\lambda = 100 (f_t - f) / (f_t - 64 / Re)$$

(f , friction factor for the sample solution; f_t , fanning friction factor for tap water), gradually decreased from 80 to 65 over a test period of approximately 1 h (ref. 4).

Lift, drag, and pressure distribution at the mid-plane were measured at the angle of attack settings of 0, 3, 6, 9, and 12 degrees at solution concentrations of 0, 1, 5, 25, 50, and 100 p.p.m.

The results are shown in Fig. 1 for a representative Reynolds number of 2×10^5 . As can be seen, the lift consistently decreases with increasing concentration. At all Reynolds numbers from 5×10^4 to 3×10^5 , even a concentration as small as 1 p.p.m. produced a reduction in lift. Also shown in Fig. 1 is the drag coefficient. At small angles of attack and within the range of concentrations tested, the drag coefficient remained nearly identical to that for water. At larger angles of attack, polymer solution flow yielded relatively larger drag coefficients. Thus it is apparent that the polymer (here we have to limit our conclusions to 'Polyox WSR-301') significantly decreases, relative to water, the lift-to-drag ratio of the foil tested.

The pressure measurements revealed relatively large amplitude oscillations particularly at the suction side of the foil. Flow visualization studies with dye (injected at various pressure taps) showed the existence of a large scale instability in the boundary-layer flow.

Although the foregoing results are based on a single type of hydrofoil, they are sufficiently consistent to tentatively conclude that the performance of hydrofoils is not likely to be improved by means of polymers. A similar conclusion has been arrived at by Wu⁴ and Kowalski⁵ in connexion with the effect of additives on propeller performance.

The reasons for the observed reduction in lift are not as

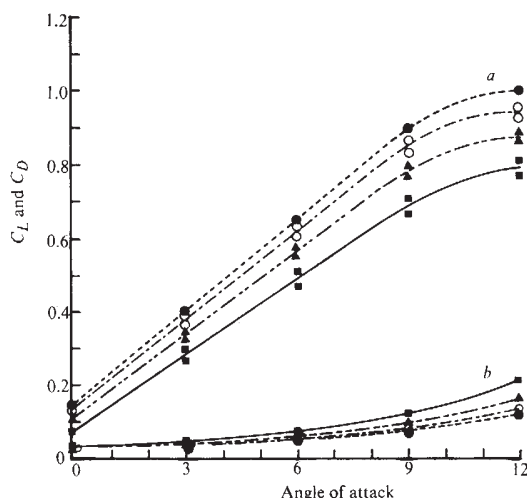


Fig. 1 Lift (a) and drag (b) coefficients as a function of the angle of attack. ●, Water. ○, 1 p.p.m.; ▲, 5 p.p.m.; ■, 100 p.p.m. polymer concentration. $C_L = 2L/\rho AU^2$, $C_D = 2D/\rho AU^2$. L , Lift force; D , drag force; ρ , density; A , lifting surface area; U , velocity of ambient flow. Both coefficients are corrected for the tunnel-wall constraint.

yet clear. Because the density and viscosity of the fluid are practically unaltered and the experiments are conducted at identical ambient flow velocities, the circulation about the foil must have been reduced by the polymer by some unknown mechanism. Finally, the large amplitude oscillations in wall pressure suggest that the polymer has a destabilizing effect on the external flow about bluff bodies. This latter conclusion is fairly well substantiated by extensive experiments carried out with circular cylinders in homogeneous polymer solutions at comparable concentrations and Reynolds numbers⁶.

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Spherical Particles formed in Rolling Contact Fatigue

IN an investigation of fracture phenomena associated with rolling contact fatigue failure¹, it was suggested that spherical particles found on fractured bearing ball surfaces could be formed from tongues of metal removed by a cavitation erosion process due to the application and release of extreme pressure in the lubricant entrapped in the propagating crack by rolling contact. A correlation between cavitation erosion and rolling contact fatigue resistance has been established². Loose pieces of metal could be severely worked by the pressure build-up in the lubricant entrapped in the fatigue crack or by pressure on the fracture faces during passage of the crack over the mating bearing surface. The mechanism of

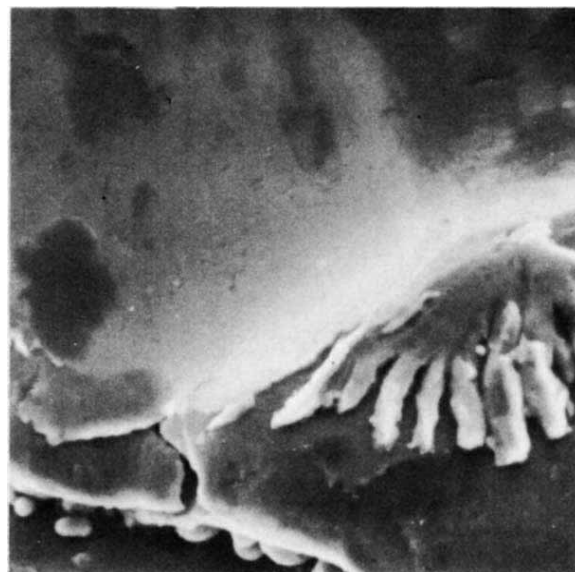


Fig. 1 Tongues of metal and associated spherical particles in the trailing edge of a surface pit, $\times 2,000$.

formation of the spherical particles and the possibility of their being artefacts^{3,4} are uncertain. It has been reported⁵ that similar spherical particles of debris are formed under specific conditions of fretting, and their formation by a process of deformation has been provided by scanning electron microscopy.

In the development of the Ferrograph⁶ as a diagnostic tool for detecting the rise of abnormal wear conditions in jet engines, it was reported that Ferrograms made from oil taken at intervals from a jet engine, in which the main rolling bearing eventually failed, revealed the presence of lathe-like turnings and spherical particles just before failure. These findings have now been confirmed in many other instances (V. C. Westcott, private communication).

Further investigation of failed balls from accelerated service-simulation rolling contact fatigue tests has confirmed the presence of tongues of metal in the trailing edge of surface pits near the end of the fracture path and spherical particles associated with them (Fig. 1), and revealed evidence



Fig. 2 A detached tongue of metal in process of being worked into a ball in the cavity below an undetached tongue of metal on the fracture surface, $\times 4,500$.

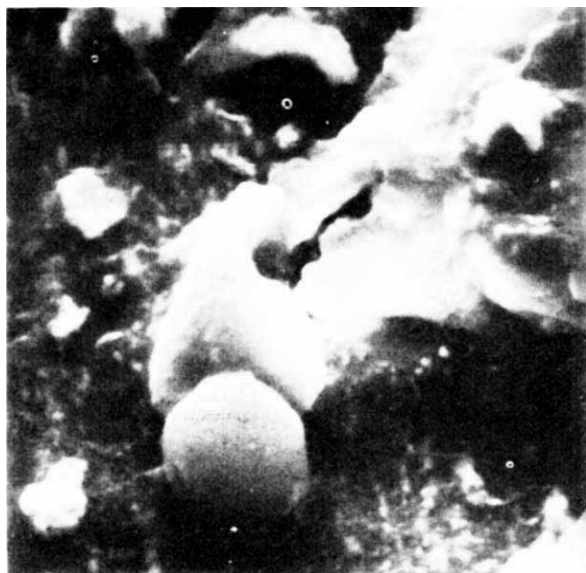


Fig. 3 A spherical particle and a partially formed spherical particle in debris from a jet engine lubricant, $\times 3,100$.

of spherical particles in process of formation by deformation of detached tongues of metal. Fig. 2 shows a detached tongue of metal being rolled into a ball in the cavity below an undetached tongue of metal on the fracture surface.

Ferrograms have also been provided⁶ for study. Scanning electron microscopical examination of Ferrograms prepared from the lubricant of a jet engine, which subsequently showed incipient fatigue failure of the main rolling bearing, revealed tongue and spherical particles of debris and evidence of the formation of spheres from the tongues of material. Fig. 3 shows a spherical particle of debris and behind it a spherical particle in process of formation from a tongue of metal. Fig. 4 shows the layer-like structure typical of spherical particles of debris, which is indicative of the mechanism of formation-rolling or balling-up of thin tongues of metal. Analysis of the spherical particles by X-ray energy analysis in the scanning electron microscope and micro-probe analysis confirmed their composition to be that of the material of the rolling bearings.

The presence of spherical particles in lubricating oil, quickly and easily revealed by the Ferrograph and confirmed

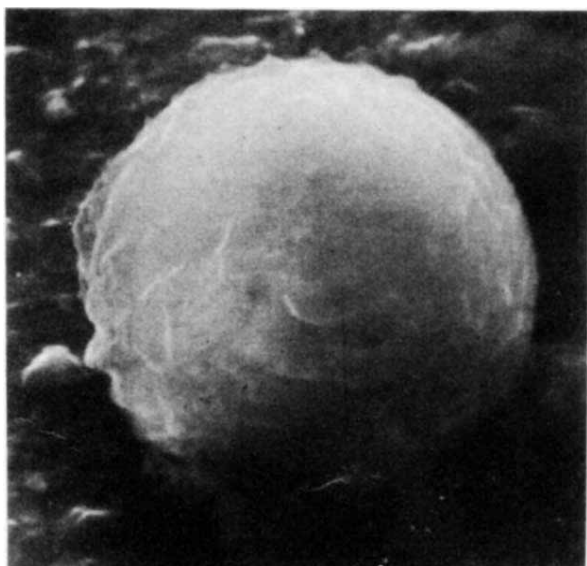


Fig. 4 Typical spherical particle of debris in aero-engine oil showing layer-like structure indicative of formation by rolling or balling-up of material, $\times 7,000$.

by electron microscopy and determination of their composition by micro-probe analysis, can form the basis of a powerful diagnostic tool for detection of distress in critical rolling bearing applications and thus prevent catastrophic failure and allow timely maintenance.

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Diamond Synthesis with Non-conventional Catalyst-solvents

THE conventional catalyst-solvents used in the high-pressure, high-temperature synthesis of diamonds from graphite are the group VIII metals, Cr, Mn, Ta, and their alloys^{1,2}. A number of other substances have been claimed to act as catalyst-solvents for the graphite-to-diamond reaction, but at the extremely high pressures and high temperatures involved, contamination by the known group VIII catalysts used in the construction of the high-pressure cells may have promoted the diamond-forming reaction. It has been demonstrated experimentally that at sufficiently high static pressures and temperatures graphite will transform spontaneously to diamond³⁻⁶ but the pressures and temperatures are about twice those required for the reactions aided by the conventional catalyst-solvents. Thus substances which may be sub-marginal as catalyst-solvents at the usual diamond synthesis pressures and temperatures might become fairly effective at higher pressures and temperatures^{7,8}.

Processes have been described which use substances such as Cu, HgO, PbO, and CaO as catalyst-solvents at pressures of 80 to 90 kbar, and temperatures of 1,800 to 2,000° C⁹⁻¹¹, instead of the usual 50 to 55 kbar, 1,300 to 1,500° C. Because earlier experiments had given negative results with Cu, Ag, and Pb at the usual pressures and temperatures, studies of such substances as catalysts at higher pressures and temperatures might resolve the role of a "catalyst-solvent" in promoting the reaction of graphite to diamond.

There are two major theories of the mechanism of diamond formation. First, only the solubility of carbon in a substance determines its efficiency in promoting the graphite-to-diamond reaction. Second, because a number of substances in which carbon is slightly soluble at favourably high temperatures and pressures yield only graphite, and even with conventional catalyst-solvent materials at pressures and temperatures definitely in the "diamond stable region", graphite and diamond crystals can nucleate and grow together, it is suggested that there may be a real difference among carbon solvents in respect of their ability to nucleate diamond; or if a diamond is already present, to nucleate new layers of diamond growth on the diamond surface.

I used a "medium high compression" belt apparatus, capable of 100 kbar pressure, which has been used very successfully for many years; the pressure and temperature characteristics have been well established by a large number of calibration experiments. In the present series all the experiments were run at 85 to 95 kbar pressure. In this apparatus the pressurized region was about 1.25 cm in diameter and 0.80 cm thick.

Two types of cells were used. Fig. 1a shows a "directly-heated" arrangement used for the first Cu experiments. The

reaction zone consisted of a sandwich of three sheets of Cu with two slabs of spectroscopic rod graphite between them. Heating current was directed to the sandwich by several copper wires at each end, the set of electrode wires at one end going to the top piston and the set at the other end to the bottom piston. The cell was of pyrophyllite. Melting of the copper in the sandwich was indicated quite clearly by a rapid increase in the cell resistance. While the Cu adjacent to the pyrophyllite could be contaminated to some extent by the pyrophyllite, the Cu in the middle was protected by the graphite, giving clean reaction conditions in the hottest part of the cell.

Fig. 1*b* shows the indirectly-heated cell which was designed to have particularly good mechanical and electrical integrity and the best possible chemical cleanliness.

To establish the temperature versus heating power characteristics of the indirectly-heated cell a preliminary experiment was run with a chromel/alumel thermocouple in place in the reaction zone. The pressure correction to chromel/alumel thermocouple readings is known to be negligibly small¹². Fig. 2 shows the calibration results carried out at pressures of 45 and 82 kbar

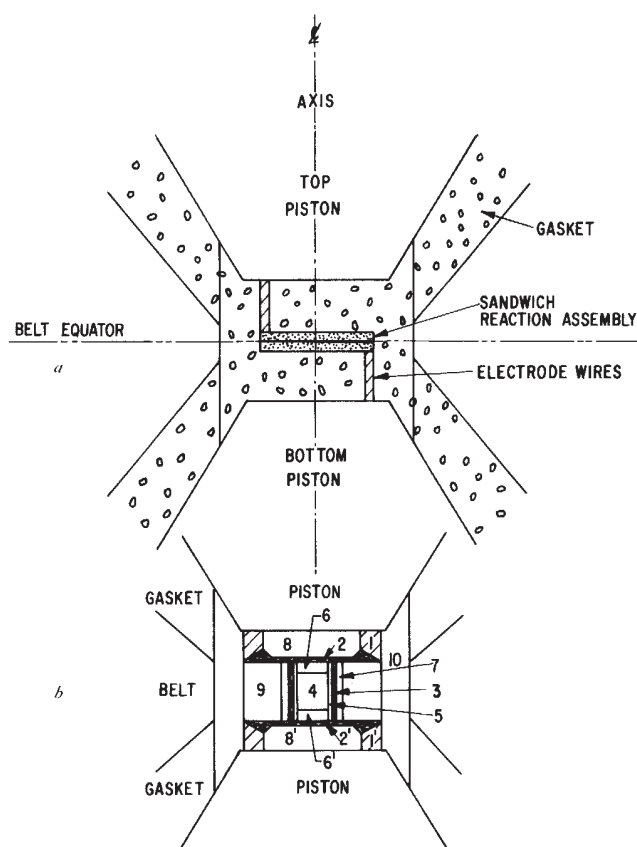


Fig. 1 Cells used in diamond formation experiments. *a*, Section of cell using directly-heated sandwiches of graphite and catalyst metal. *b*, Section of cell with isolated reaction zone (4) indirectly heated by a graphite heater sleeve (3). The heating circuit consisted of the steel current rings 1, 1', the graphite current disks 2, 2', and the graphite sleeve heater 3. The reaction zone 4 was inside the graphite heater and isolated from it by the Al_2O_3 sleeve 5 and the Al_2O_3 disks, 6, 6'. The hot zone just outside the graphite heater sleeve was occupied by the Al_2O_3 sleeve 7. The two disks 8, 8', and the sleeve 9 were made of pyrophyllite stabilized by prebaking in air at about 800° C. The containing sleeve, 10, and the gaskets were of untreated pyrophyllite. Reaction materials were made up in the form of pills of disks and inserted into the space 4. When the reactants were initially powders they were prepressed into right circular pills in a clean pressing mould to bring them to approximately theoretical density before insertion in the high pressure cell. Metal components were punched out as disks from sheets of the desired metal. The reaction chamber, 4, was 0.20 cm diameter by 0.25 cm long. Before test runs parts were stored in a vacuum desiccator at 110° C to minimize absorbed water.

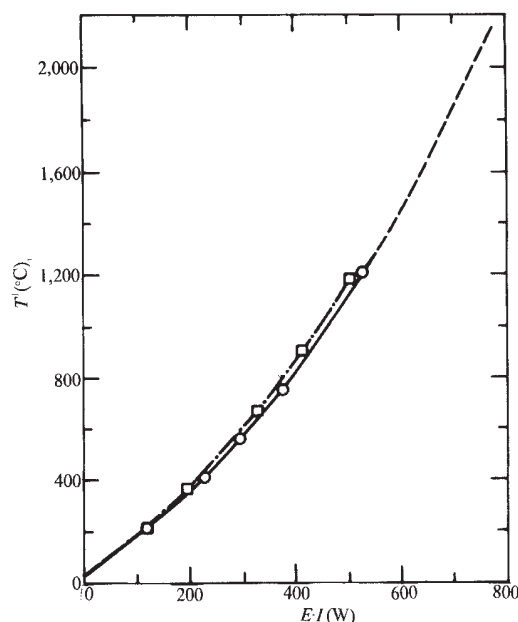


Fig. 2 Temperature/heating power calibration of the cell shown in Fig. 1*b*. $\square$ — $\square$, 45 kbar; $\circ$ — $\circ$, 82 kbar; ---, extrapolation to 2,000° C.

up to the reliable limit of chromel/alumel—about 1,200° C. The cell materials were stable and clean enough at higher temperatures so that the chromel/alumel calibration could be extrapolated to 2,000° C with reasonable confidence. In test runs with a Pt catalyst the power required confirmed the extrapolation.

After runs were made the contents of the reaction zones were examined by optical microscopy, by sapphire scratch testing, and by X-ray diffraction. Operator experience makes such techniques very discriminating.

The first tests on metals were made with the directly-heated cells. The first test was made with Ni which gave the fast, complete, conversion of graphite to diamond expected. Second tests were with Pt, which has a melting point of roughly 2,000° C at the pressure used. Once melted, and reacting, the Pt/graphite cells tended to “run away” in terms of power consumption resulting in overheating and loss of any diamond formed initially. This happened in two runs but subsequent runs, kept under control, gave good diamond yields. Oxygen-free high-conductivity (OFHC) copper was then substituted for Pt and runs were made at the same power levels. The copper melted but gave no reaction at all with the graphite. As a final test of this cell and method, the metal parts were replaced by an alloy of 99 Cu 1 Ni which had been tested at 55 kbar and would not yield diamond⁷. At the higher pressure this alloy formed fine-grained, white diamond rapidly at the areas where melting occurred.

With indirectly-heated cells tests were made using, successively, Pt, PbO , Cu, HgO , CaO, 99 Cu 1 Ni, and 95 Cu 5 Ni at temperatures in the 1,500° C to 2,000° C range. The cells were stable under these conditions. The Pt, 95 Cu 5 Ni, 99 Cu 1 Ni and Cu catalyst-solvents were inserted as disks in the reaction zone, with disks of graphite interspersed between them. Oxide catalysts were used in two ways: (i), powder mixes of the oxides with graphite were prepressed into pills and inserted as such, and (ii), prepressed pills of the oxide and pills of graphite were inserted alternately to form sandwich layers.

The Pt and 95 Cu 5 Ni catalyst experiments yielded diamond readily in this cell, whereas the 99 Cu 1 Ni was marginal with some runs giving small yields and some none. None of the pure Cu runs reacted with the graphite.

The oxide catalyst experiments yielded no diamonds. The oxides of Hg and of Pb decomposed to the metallic element under the conditions of reaction, presumably by carbon

reduction of the oxides. In the CaO experiments there was no evidence of reaction. The graphite may have become better graphitized and more slippery as a result of the heating at high pressure in the presence of the CaO.

I conclude that some other conditions—possibly contamination by some other catalytic materials—are necessary to make the graphite–diamond reaction proceed, even at the advanced pressures and temperatures, when Cu, PbO, HgO, or CaO are used as parts of the reaction system.

My experiments using 99 Cu 1 Ni, in which diamond formed, showed that very small additions of conventional catalyst metals are enough to promote diamond formation. The solubility of carbon in this very dilute alloy of Ni in Cu is lower than that in Pb⁷. Because Pb showed no evidence of diamond formation it appears that small amounts of Ni in a Cu alloy, both provide a carbon solvent, and promote diamond nucleation. These higher pressure experiments substantiate the idea that a catalyst-solvent substance for the graphite-to-diamond reaction must be both a carbon solvent and a diamond nucleant.

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BIOLOGICAL SCIENCES

Evolution of Enzyme Regulator Sites: Evidence for Partial Gene Duplication from Amino-acid Sequence of Bovine Glutamate Dehydrogenase

MODULATION of the biological activity of proteins by metabolite levels is a widespread and well-documented phenomenon. As Monod, Wyman and Changeux¹ pointed out, most regulatory protein molecules are composed of several subunits. The regulation of some oligomeric proteins, notably haemoglobin², involves interaction of similar sites on separate similar subunits. In other cases such as that of aspartate transcarbamylase³ there are separate catalytic and regulatory subunits. Some proteins, however, possess within individual subunits both active sites and regulatory sites capable of mediating homotropic and/or heterotropic interaction.

Glutamate dehydrogenase (GDH) from the liver of vertebrates is such a protein. Its catalytic activity is modulated by the coenzymes of the reaction and by such purine nucleotides as ADP and GTP⁴⁻⁷, and yet the hexameric enzyme molecule contains only one type of polypeptide chain⁸. The precise relation between the purine nucleotide sites and the coenzyme binding sites remains uncertain, but it has been shown clearly

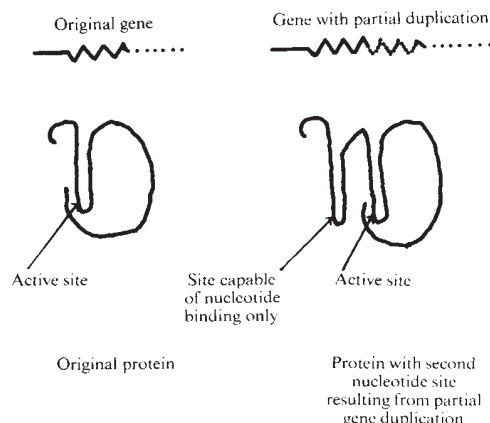


Fig. 1 Schematic diagram illustrating the possible partial gene duplication and its consequences in terms of amino-acid sequence, protein structure and enzyme function.

that the enzyme can bind more than one molecule of NADH per subunit^{4,9-12}. It appears that binding at the second site may account for inhibition by high levels of NADH¹³.

If one assumes that in the course of enzyme evolution catalytic activity preceded the fine control imposed by regulatory sites, the GDH found in vertebrate liver must be descended from an ancestral monomeric dehydrogenase possessing the active but not the regulatory site. It is interesting, therefore, to consider the possible evolutionary origin of the regulatory site.

There are two basic possibilities:

(1) Gradual mutational modification of the genome resulting ultimately in the independent formation of a second, regulatory site, a process analogous to the original evolution of the active site. (2) The incorporation, by unequal crossing-over, for example, of part of a pre-existing gene. In the special case where the regulator is identical with or structurally similar to one of the substrates this would be most simply achieved through partial duplication of the structural gene for the enzyme itself. This would result (Fig. 1) in a protein containing a repeated sequence of amino-acid residues in its molecule.

The disadvantage of the first possibility is its inherently low probability¹. The second is open to the objection that neither of the duplicated sequences would be in an intramolecular environment identical to that of the ancestral sequence. The protein might therefore fail to fold properly, resulting in obliteration rather than duplication of function. Such a protein would, of course, be discarded by natural selection. Even if the duplicated sequences folded correctly, full function might depend upon participation of other residues which would be accessible to only one of the duplicated sites (Fig. 1). Nevertheless, even if one site retained only a substrate-binding capacity, it could evolve a regulatory function, provided that the other site retained full functional activity. This method offers an evolutionary short-cut and appears more likely than the other.

Although regulatory proteins have not been examined from this standpoint, there are documented cases of duplication having occurred within a single structural gene. Bacterial ferredoxin molecules consist of two clearly related halves¹⁴⁻¹⁷, while those of the heavy chain of gamma G immunoglobulin contain three similar portions¹⁸. There is also evidence for two gene duplications in the evolution of papain¹⁹.

The nucleotide sequence coding for the potential regulatory site, once established, would mutate independently and probably not conservatively. "Paralogous" sequences as defined by Fitch and Margoliash²⁰ (human myoglobin and α haemoglobin) tend to diverge more than "orthologous" sequences (human α and β haemoglobins). The duplicated sequences in regulatory proteins envisaged above may be regarded as a special case of paralogous sequences within the

Fig. 2 Comparison of suitably aligned partial sequences of beef liver GDH and pig muscle glyceraldehyde-3-phosphate dehydrogenase. The uppermost of the three sequences shows residues 198–261 of the porcine dehydrogenase. The middle sequence covers residues 267–330 of beef liver GDH, designated the “regulator” sequence in the text. The lower sequence is that of residues 112–175 of the bovine enzyme and includes the active site lysine, Lys 126. The homologies are indicated in capital letters. The GDH sequences are taken from the results of Moon, Piszkiwicz and Smith²¹, while the glyceraldehyde-3-phosphate dehydrogenase sequence is that reported by Harris and Perham²³.

G3PDH	GLY	ALA	Ala	Glu	Asn	Ile	Ile	Pro	Ala	SER	Thr	GLY	Ala
GDH2	GLY	ALA	LYS	CYS	Val	Ala	VAL	Gly	Glu	SER	Asp	GLY	Ser
GDH1	Thr	Tyr	LYS	CYS	Ala	Val	VAL	Asp	Val	Pro	Phe	GLY	Gly
G3PDH	ALA	LYS	ALA	Val	Gly	LYS	Val	Ile	PRO	Glu	Leu	Asp	Gly
GDH2	Ile	Trp	Asn	Pro	Asp	Gly	ILE	Asp	PRO	LYS	Glu	Leu	Glu
GDH1	ALA	LYS	ALA	Gly	Val	LYS	ILE	Asn	PRO	LYS	Asn	Tyr	Thr
G3PDH	Lys	Leu	Thr	Gly	Met	Ala	Phe	Arg	Val	Pro	Thr	Pro	Asn
GDH2	ASP	Phe	Lys	LEU	Gln	His	Gly	THR	Ile	Leu	Gly	Phe	Pro
GDH1	ASP	Glu	Asp	LEU	Glu	Lys	Ile	THR	Arg	Arg	Phe	Thr	Met
G3PDH	Val	Ser	Val	Val	Asp	Leu	Thr	Cys	Arg	LEU	GLU	Lys	Pro
GDH2	Lys	Ala	Lys	Ile	Tyr	Glu	Gly	Ser	Ile	LEU	GLU	VAL	ASP
GDH1	Glu	Leu	Ala	Lys	Lys	Gly	Phe	Ile	Gly	Pro	Gly	VAL	ASP
G3PDH	Ala	Lys	Tyr	Asp	Asp	Ile	Lys	Lys	Val	Val	LYS	GLN	
GDH2	Cys	Asp	Ile	Leu	Ile	Pro	Ala	Ala	Ser	GLU	LYS	GLN	
GDH1	Val	Pro	Ala	Pro	Asn	Met	Ser	Thr	Gly	GLU	Arg	Glu	

same protein. In this special case one might expect selective pressures to produce considerable divergences in those parts of the duplicated sequences concerned only with maintaining the structure of the protein.

It was of great interest to search the sequence of bovine liver GDH^{8,21} for the vestigial homologies predicted on the basis of the hypothesis above. Two extended sequences in the protein subunit (similar in chicken GDH)²¹ show considerable homology. If residues 114–163 are aligned with residues 269–318, no less than 12 of the 50 residues are in identical positions (Fig. 2). The homology includes a Lys-Cys sequence (114, 115) and a Pro-Lys sequence (134, 135) and is greatest in the vicinity of these two sequences. The intervening twelve residues show only one homology. This stretch includes Lys-126 which reacts with pyridoxal phosphate and is thought to be essential for catalytic activity²². In the homologous sequence this essential lysine is replaced by tryptophan and there are several other markedly non-conservative changes.

In addition to 24% of their residues in identical positions, the two sequences contain a further 38% that could have resulted from single base changes in the nucleotide sequence.

If one takes two completely random sequences each n residues in length and composed of the twenty common amino-acids, the probability of the occurrence of x and only x homologies is

$$\frac{n! 19^{n-x}}{x! (n-x)! 20^n}$$

From this it can be calculated that the probability of 1, 2 or 3 homologies is quite high (0.21, 0.27 and 0.23) but the probability dwindles rapidly for greater numbers of homologies and is 4.3×10^{-6} for 12 homologies. In the GDH sequence, however, there are 352 possible sequences of 50 residues with which to match the sequence in question, if overlapping pairs of sequences are ignored. The probability that one of these sequences will contain 12 homologies with the chosen sequence is therefore $352 \times 4.3 \times 10^{-6}$ ($= 1.5 \times 10^{-3}$), as $4.3 \times 10^{-6} \ll 1$. This is an approximation, as no account has been taken of the differing frequencies of different amino-acids.

It seems likely therefore that the homology between residues 114–163 and 269–318 of beef liver GDH reflects common ancestry. Convergent evolution seems most unlikely in this case in view of the marked differences between sequences 119–130 and 274–285. These differences probably reflect the divergence predicted above. Convergent evolution might in any case be expected to produce homologies less mathematically precise and more qualitative in nature.

Smith *et al.*⁸ have compared the sequence of bovine GDH with that of glyceraldehyde-3-phosphate dehydrogenase from pig muscle²³, and conclude that there is very little homology, with the exception of the sequence immediately surrounding the active site lysine (Lys 126) of GDH. Residues 209–219 of glyceraldehyde-3-phosphate dehydrogenase and 123–133 of GDH have six residues in identical positions in both sequences.

It seemed possible that the “regulator” sequence of GDH might retain homologies with the glyceraldehyde-3-phosphate dehydrogenase sequence that had been lost in the “active site” sequence. Accordingly in Fig. 2 the glyceraldehyde-3-phosphate dehydrogenase sequence is also aligned for comparison. The comparison, summarized in Table 1, shows that outside the region comprising residues 209–219 the glyceraldehyde-3-phosphate dehydrogenase sequence actually shows considerably greater homology with the regulator sequence than with the active site sequence of GDH. Twenty of the 64 residues in the regulator sequence are identical with the corresponding residues in at least one of the other sequences.

Table 1 Comparison of Homology of Glyceraldehyde-3-phosphate Dehydrogenase with “Active Site” and “Regulator” Sequences of Beef Liver GDH

	Identical residues	Alterations explicable by single base changes	Alterations requiring 2 or 3 base changes
G3PDH sequence and matching GDH “active site” sequence	6	30	28
G3PDH sequence and GDH “regulator” sequence	9	21	34
GDH “active” site sequence and GDH “regulator” sequence	12	28	24

The sequences used in this comparison are those shown in Fig. 2.

The evidence for an evolutionary relationship between the three sequences is persuasive. Divergence from an ancestral enzyme probably led separately to glyceraldehyde-3-phosphate dehydrogenase and to a primitive GDH lacking the regulatory site and possessing a subunit considerably smaller than that of beef liver GDH. Later, a partial gene duplication probably resulted in a protein in which a sequence of about 155 residues containing at least part of the active site was repeated. One of the identical sequences must have remained catalytically functional. If the other sequence retained its nucleotide-binding capacity it could then evolve as a regulatory site.

At present no other GDH sequences are available. It is significant, however, that most vertebrate GDHs studied so far are regulated by nucleotides and have molecular weights of about 330,000; the corresponding NADP-linked enzymes from yeast, *E. coli* and *Neurospora* are not regulated by nucleotides and available values for the molecular weights of GDH from lower organisms are all in the region of 250,000⁴. One may predict that when sequences become available for other higher organisms they will further document the partial gene duplication discussed above. The non-regulatory GDH from lower organisms, on the other hand, may well lack the duplicated sequence of 155 residues.

In view of the importance of Lys 126 in the active site sequence and its absence from the corresponding position of the presumed regulator sequence it is most interesting to note that Wallis and Holbrook²⁴ have shown that the chemical modification of this residue abolishes the binding of 2-oxoglutarate but not the binding of coenzymes. It is likely therefore, as required by the basic hypothesis developed in the present paper, that the replacement of lysine by tryptophan in the corresponding position of the regulator sequence, while abolishing any possible catalytic activity due to that sequence, would leave the nucleotide binding capacity intact.

The present study appears to represent the first evidence bearing upon the evolution of regulatory sites from ancestral catalytic sites. It remains to be seen whether similar mechanisms have operated in the evolution of other regulatory enzymes.

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Mapping the DNA Fragments produced by Cleavage of λ DNA with Endonuclease RI

THE analysis of *in vitro* DNA-directed transcription and coupled transcription/translation is usually complicated by the very large number of products that are made from each DNA molecule. If smaller DNA molecules could be used, the analysis would be correspondingly easier. The discovery of several restriction endonucleases, each recognizing a small number of unique cleavage sites per DNA molecule, could lead to a much deeper analysis of the complex structure and function of DNA by making it possible to isolate homogeneous DNA segments of manageable size. One of these enzymes, the RTF RI endonuclease, has recently been characterized by Yoshimuri, who has shown that it introduces a limited number of cuts in λ DNA¹; by sucrose gradient sedimentation Yoshimuri separated a faster-moving peak containing one unique DNA population of molecular weight 13×10^6 from a slower-moving peak consisting of smaller fragments of average molecular weight 3.8×10^6 . We describe here the further resolution of the products arising from digestion of linear λ DNA with the RI endonuclease into six discrete fragments, and their subsequent characterization and mapping along the λ genome.

When the products of digestion of wild type λ DNA with RI endonuclease are submitted to polyacrylamide gel electrophoresis as described in the legend to Fig. 1, six bands result.

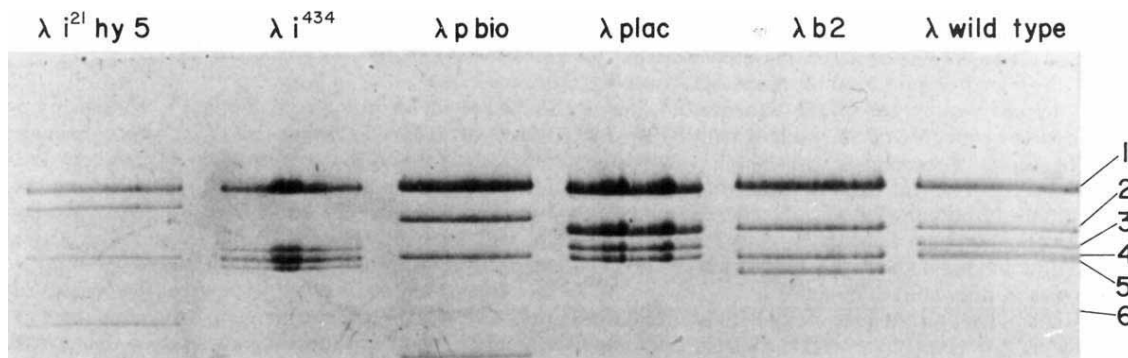


Fig. 1 Polyacrylamide gel separation of the endonuclease RI fragments obtained from various λ DNA species. The phages were grown and the DNA extracted as previously described². The RI endonuclease was purified from an RTF carrying *E. coli* B strain according to Yoshimuri¹, and titrated against λ DNA to determine the amount of enzyme needed to make the reaction go to completion. The DNA was digested with RI endonuclease for 30 min at 37° C in 90 mM Tris-HCl, pH 7.5, 10 mM MgSO₄. The reaction mixture was then extracted with phenol saturated with 10 mM Tris-HCl, pH 8.0, 1 mM EDTA, at 4° C, and the aqueous phase was precipitated with 2 volumes of ethanol at -20° C. The samples (8-15 μ g of DNA) were resuspended in 5-10 μ l. of electrophoresis buffer (see below) containing 10% sucrose, and 0.02% bromophenol blue as a marker, before being loaded into the sample wells of the gel. The separating gel was a 0.3 cm $\times$ 12.5 cm $\times$ 12.5 cm slab containing a linear concentration gradient of polyacrylamide, from 2.5% at the origin to a final concentration of 7.5%. (Details of the preparation of the gels will be published elsewhere—P. G. N. J., manuscript in preparation.) The buffer used for the gel and reservoirs was 40 mM Tris acetate, pH 8.3, 20 mM sodium acetate, 2 mM EDTA. Electrophoresis was carried out with a current of 45-50 mA for 20 h at room temperature. After the electrophoretic run, the DNA was stained by soaking the gel with a 0.02% solution of methylene blue for approximately 2 h, and then the excess stain was removed by washing the gel with several changes of water. (The methylene blue stained DNA was kept out of strong light to minimize the possibility of photoactivated degradation of the DNA.)

Bands 1, 2 and 3 are well separated. Two other bands run very close together, forming a doublet (bands 4 and 5). The fastest-migrating segment appears to be present in less than stoichiometric amounts (band 6), as indicated by the relatively low intensity of the band seen at this position; the reason for this is discussed below. Each of the bands was eluted from the gel and samples were prepared for electron microscopy length measurements by means of the Kleinschmidt spreading procedure^{3,4} (see legend Fig. 2). This permitted an estimation of the molecular weights of the fragments, which are shown in Table 1: the sum of the molecular weights of the fragments agrees closely with the molecular weight estimated for total λ DNA, indicating that together they account for the whole λ DNA molecule. This, in turn, suggests very strongly that each is a unique homogeneous DNA species. Table 1 shows that the distance migrated on the gel is inversely related to the molecular weight determined for each of the fragments, except that 3 and 4, which are well separated by electrophoresis at room temperature (Fig. 1), are found to have identical molecular weights. We have found that if a similar electrophoretic separation is made at 4° C (not shown here) the fragments corresponding to 3 and 4 migrate together. The possible significance of this finding will be discussed below.

As pointed out earlier, fragment 6 is apparently produced in less than stoichiometric amounts. The reason for this became evident from the electron micrographs of DNA extracted from band 1 of the gel (Fig. 2). In some cases there was a small DNA piece, with the same length as a typical segment 6 molecule, lying near the end of the fragment 1 molecule, as if at the last moment the act of spreading for electron-microscopy had separated them. We therefore conclude that fragments 1 and 6 are the two termini of the λ DNA molecule, and that the two frequently migrate together at position 1 in the gel, bound together through the complementary single-stranded regions located at both ends of the total λ DNA molecule⁵, thus accounting for the low recovery of pure segment 6 at the faster position on the gel.

The availability of a wide range of well characterized deletion, substitution, and insertion derivatives of phage λ (for an extensive review, see ref. 6) made it possible to compare the gel patterns obtained by digesting wild type λ DNA and some of the derivatives. Fig. 1 shows such an analysis, comparing wild type λ DNA with $\lambda b2$, $\lambda plac5$, $\lambda db30-7 nin5$ (which we shall refer to here as $\lambda pbio$), $\lambda i434$, and $\lambda i21 hy5$ DNAs. A comparison of the bands present, absent or changed in position for the different mutants with respect to the wild-type pattern, together with the electron microscopy data presented above, allows us to establish the order of the fragments, as shown in Fig. 3.

$\lambda b2$ is deleted between positions 45.3 and 57.4 of the map (see Davidson and Szybalski⁷, and Fig. 3); the band pattern of this mutant lacks fragments 3 and 4 but shows instead a faster-moving piece. This result indicates that fragments 3 and 4 are adjacent, and that the RI recognition site that

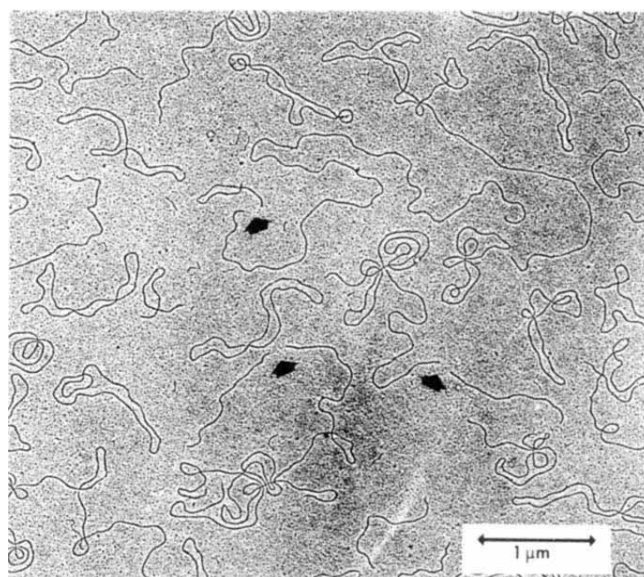


Fig. 2 DNA eluted from position 1 of wild type λ DNA (see Fig. 1) and spread with cytochrome *c* according to the techniques described in Table 1. The arrows point to gaps between short terminal fragments and long terminal fragments of λ DNA, which were pulled apart during the spreading with cytochrome *c*.

separates them (and is absent in $\lambda b2$) is located between map positions 45.3 and 57.4.

$\lambda pbio$ has been studied extensively by Fiandt *et al.*⁸ and contains two alterations of the wild type λ genome: (1) the DNA between map positions 56.5 and 77.5 has been deleted, and instead some genes of the *bio* operon have been inserted, corresponding to 9.3 λ units in length; (2) a further deletion occurs between map positions 83.8 and 89.2. The band pattern for this mutant shows that wild-type DNA segments 2, 3 and 5 have been replaced by a slow-migrating band between wild-type fragments 1 and 2, and a band migrating faster than wild-type fragment 5. Hence it can be deduced that, as fragment 4 appears unaltered in the gel pattern, the RI recognition site between wild-type segments 3 and 4 must lie outside the 56.5–77.5 $\lambda pbio$ deletion, and this site must be to the right of segment 4; furthermore, both segment 2 and segment 5 must lie to the right of segment 3.

The single remaining piece of evidence necessary to complete the ordering of the fragments comes from the band pattern obtained with $\lambda i434$. This mutant differs from wild type λ in its immunity region, which is smaller than and mainly nonhomologous to the λ immunity region⁸. As the fragment corresponding to wild type segment 2 is the only one missing from this mutant, being replaced by a fragment migrating just below fragment 5, the immunity region must

Table 1 Molecular Weight Determination of the Fragments

	Total λ	1	2	3	4	5	6
No. of molecules measured	16	13	16	28	28	22	30
Average lengths (μ m)	16.80	7.79	2.68	2.11	2.07	1.75	1.26
Standard deviation (μ m)	± 0.36	± 0.24	± 0.092	± 0.051	± 0.042	± 0.049	± 0.027
MW $\times 10^6$	30.85	13.69	4.49	3.54	3.54	3.00	2.31

* Calculated by comparison with PM2 DNA (molecular weight = $6.40 \times 10^6 \pm 2\%$) used as internal standard.

Bands of wild type DNA fragments, separated as shown in Fig. 1, were cut out from the gel (fragments 4 and 5 were taken together). The gel pieces were transferred into plastic tubing, and their DNA content was collected electrophoretically into dialysis tubing immersed in the lower reservoir of the electrophoresis apparatus (12 h at room temperature, 50–100 V). The buffer for this elution was the same as that described in Fig. 1. The DNA was then concentrated by ethanol precipitation followed by resuspension in 10 mM Tris, pH 8, 1 mM EDTA. The spreading of the DNA with cytochrome *c* and 30% formamide was done as described earlier^{3,4}.

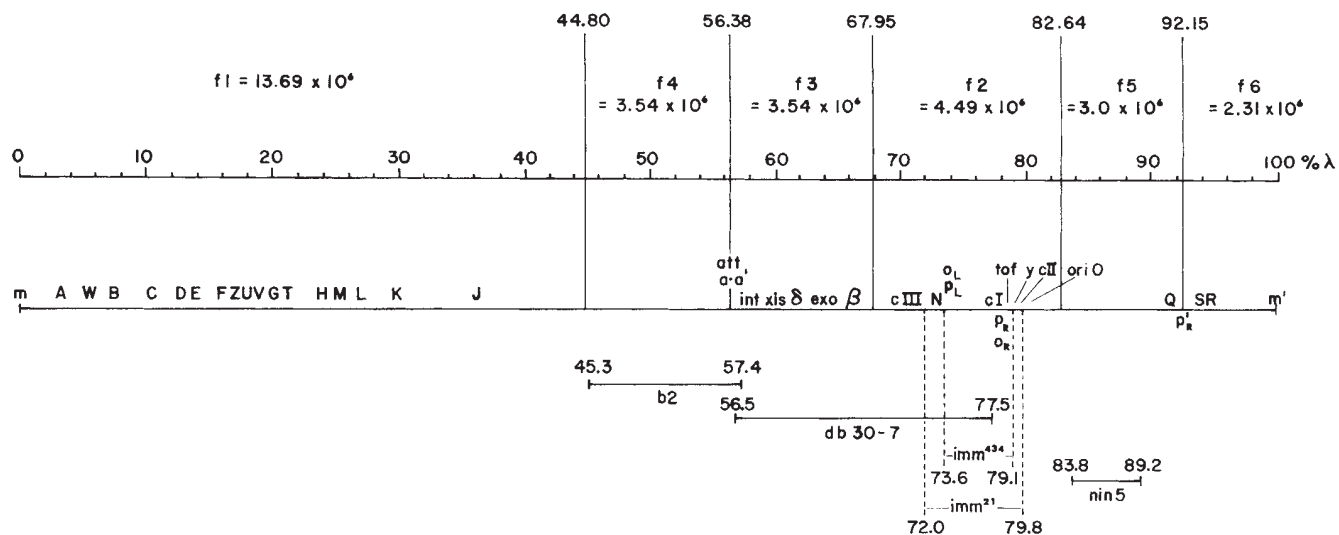


Fig. 3 Position of the six λ DNA fragments along the whole molecule. The positions of the RI endonuclease cleavage sites, as established by combined electron microscopy and gel analysis data, are indicated on the λ DNA map (adapted from Davidson and Szybalski⁷, with permission). We assume that the total $\div$ scale represents the sum of the molecular weights found for the six fragments, that is, 30.57×10^6 daltons. This value agrees closely with that estimated by Davidson and Szybalski⁷ ($30.8 \pm 1 \times 10^6$), and also with our measurement of the total λ DNA (30.85×10^6) (Table 1).

be located in segment 2. There is also a new, very fast-migrating band in the λ i434 pattern, probably arising from a further RI recognition site within the λ i434 nonhomologous immunity region.

The electron microscopy data combined with the mutant analysis lead to the order of fragments shown in Fig. 3. Molecular weight considerations dictate that segment 1 is the left-hand terminus and segment 6 the right-hand terminus of the λ DNA molecule. The two remaining mutants analysed in Fig. 1 lend confirmation to this order of fragments. Thus λ plac5 contains an insertion of the genes of the *lac* operon in a region previously located somewhere between genes J and N⁹; in λ plac5, fragment 4 is replaced by a larger DNA segment migrating very close to wild type fragment 2. From Fig. 3 we can therefore locate the position of the *plac5* insertion between map positions 44.80 and 56.38, which are indeed between genes J and N (see Fig. 3).

The hybrid λ i21 *hy5* is known to contain the phage 21 immunity region and, in addition, further uncharacterized regions of nonhomology with wild-type λ . Its pattern of cleavage by the RI endonuclease suggests that it lacks the wild-type enzyme recognition sites between segments 2 and 3, and between segments 3 and 4. As a result, the hybrid possesses a large fragment migrating just faster than wild type fragment 1. The λ i21 band pattern also shows a DNA fragment of lower molecular weight than wild type fragment 6, suggesting that either (1) the corresponding region of DNA in the hybrid is smaller than in wild type λ , or (2) the RI enzyme recognizes a new site in the λ i21 immunity region. In the latter case, the absence of segment 6 from the gel pattern of the hybrid would be due to a complete adhesion of the two termini during the gel fractionation procedure. We have not investigated these possibilities further.

It should be noted that in the above argument we have assumed that if a DNA fragment from a mutant co-migrates on the polyacrylamide gel with a fragment of wild type λ DNA, then the two fragments are, except for possible minor sequence differences, identical and come from the same region of whole λ DNA. The internal consistency of the results obtained and their agreement with the known physical map of the λ DNAs employed strongly suggest that this assumption is correct.

These results show that it is possible to separate DNA fragments of molecular weights up to approximately 5×10^6 according to their size by polyacrylamide gel electrophoresis.

As the molecular weight increases, however, such separations become more difficult in this system, and DNA fragments of greater than approximately 10^7 molecular weight tend to move together in a very close band in the position of fragment 1 (unpublished observation). Moreover, at room temperature, smaller size DNA pieces may also exhibit unexpected mobilities; fragment 4, which shows a greater mobility at higher temperatures with relation to the other fragments, lies mainly in the b2 region of λ DNA, which has been shown to be low in G+C content (37%)⁶. Perhaps by virtue of this base composition, increasing temperatures render fragment 4 less rigid, and hence less resistance is offered to its motion through the gel at room temperature.

Our ability to separate the fragments produced by digestion of λ DNA by the RTF RI endonuclease simply on polyacrylamide gels, and to establish their location on the λ genome, should make it possible to characterize any strain of λ phage in which large deletions, substitutions, or insertions occur in the DNA, as illustrated above in the cases of λ plac5 and λ i21. Furthermore, the availability of homogeneous segment preparations of known regions of the whole λ DNA, such as we have described, should facilitate studies on *in vitro* transcription, coupled transcription/translation, and sequence analysis of λ DNA.

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Magnetic Resonance Studies of Deoxyribonucleoprotein

THE structure of chromatin, and the problems of organizing large lengths of DNA within the cell nucleus, are currently of considerable interest. It now appears established that the agents of chromosome structure are the histones found in conjunction with DNA in the chromosome, which may also act at some stage of genetic regulation. Of the five major types of histone fraction normally associated with mammalian genetic material, the lysine-rich histone F1 (or histone I) has some properties which set it apart from the others. It contains a very high proportion of lysine (27%), alanine (24%) and proline (9%) residues, these three alone making up more than 60% of the 216 residues of the molecule. As expected from the high proline content, F1 forms very little α -helix¹ in aqueous salt solutions; it also shows less tendency to aggregate than the other fractions. Interchain interactions, however, involving a specific section of the molecule are induced by salt^{2,3}; a proposed scheme for this interaction, involving an antiparallel alignment of chain segments, has been presented³.

Another distinguishing feature of F1 is the relative ease with which it is removed from the deoxyribonucleoprotein complex. It is completely removed by 0.4–0.5 M NaCl⁴ or by 0.1 M MgCl₂⁵ while all the other fractions remain bound to the complex, though not necessarily to the same sites⁶ at these molarities. This ready removal of F1 from an otherwise stable complex forms the basis of the N.M.R. studies to be described.

Several roles have been suggested for F1. Mirsky *et al.*⁷ have shown by electron microscopy that F1 is associated with a "clumping" of DNA fibres. Ord and Stocken⁸ have produced evidence that the phosphorylation of histones may modify their interactions with DNA, and Bradbury, Inglis, Matthews and Sarnar (unpublished results) have shown that the lysine-rich histone fraction in *Physarum polycephalum* is phosphorylated just before mitosis, at a time when chromosome condensation is taking place. These and other results suggest that the lysine-rich histone fraction found in DNP may play a large part in the high-level organization of chromatin and perhaps in the condensation of the chromosome, which is supported by our present results.

Nuclear magnetic resonance spectra were obtained, using a standard time-averaging computer, on the 'Jeol JNM-4H' 100 MHz spectrometer and on the 'Varian HR-220' spectrometer belonging to the SRC. Standard 5 mm tubes were used. No attempt has been made at this stage to compensate for changes in sample concentration consequent upon gel condensation. Simulated spectra were generated as described⁹. DNP was prepared by the method of Zubay and Doty¹⁰. The final gel was analysed by infrared spectroscopy¹¹ and always showed between 52% and 56% of protein in the complex. Partial DNP ($P_{0.6}$) was obtained

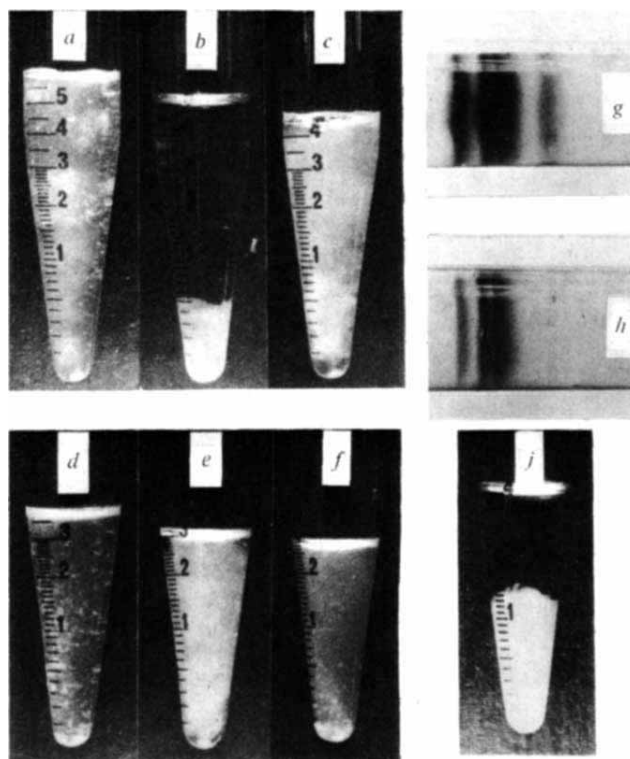


Fig. 1 Deoxyribonucleoprotein gel after spinning at 1,000g for 5 min. *a*, Total DNP in D₂O; *b*, Total DNP in 0.2 M NaCl; *c*, Total DNP in 0.5 M NaCl; *d*, Partial DNP ($P_{0.6+CMC}$) in D₂O; *e*, Partial DNP in 0.2 M NaCl; *f*, Partial DNP in 0.5 M NaCl; *g*, DNP gel containing ~7% of original histone F1, in 0.2 M NaCl; *h*, Polyacrylamide gel pattern of histones from total DNP; *i*, Polyacrylamide gel pattern of histones from partial DNP ($P_{0.6+CMC}$).

by homogenizing DNP gel with 40 volumes of 0.6 M sodium chloride, stirring for 2 h, and then pelleting at 140,000g for 3 h. Subsequent analysis of the histones in this material by the polyacrylamide gel electrophoresis technique of Johns¹² showed that 6–8% of the original F1 content remained. A more completely F1-depleted material ($P_{0.6+CMC}$) was obtained by adding carboxymethyl cellulose to the DNP homogenate at the stirring stage; the resulting partial DNP contained less than 2% of the original F1 as estimated from densitometer traces. Photographs of the polyacrylamide gels obtained from the DNP and the F1-depleted DNP are shown in Fig. 1 (*g*) and (*h*).

When the ionic strength (NaCl) of a DNP gel is increased a reversible contraction occurs (Fig. 1, *a*, *b*, *c*). These photographs are all of the same sample; in each case the sample has been dialysed overnight against 500 volumes of the stated sodium chloride solution, transferred to a centrifuge tube and spun at 1,000g for 5 min before being photographed. It can clearly be seen that at an ionic strength of 0.2 M NaCl, the gel has shrunk to about one-tenth of its original volume (Fig. 1*b*) while an increase to 0.5 M NaCl causes a complete return to the original volume (Fig. 1*c*). No such shrinkage takes place in gels from which F1 has been removed (Fig. 1, *d*, *e*, *f*). Further, the partial nucleoprotein $P_{0.6}$ containing 6–8% of its original F1 shrinks to about 35% of its original volume (Fig. 1*j*). A quantitative estimate of the shrinkage caused in total DNP by NaCl and by MgCl₂ is given in Table 1.

Gel shrinkage corresponds to a solubility minimum noted by several workers¹³. We have measured the turbidity of solutions of total and F1-depleted DNP by monitoring the absorbance at 600 nm, and find that a very large increase in turbidity is shown by total DNP over the range 0.1–0.3

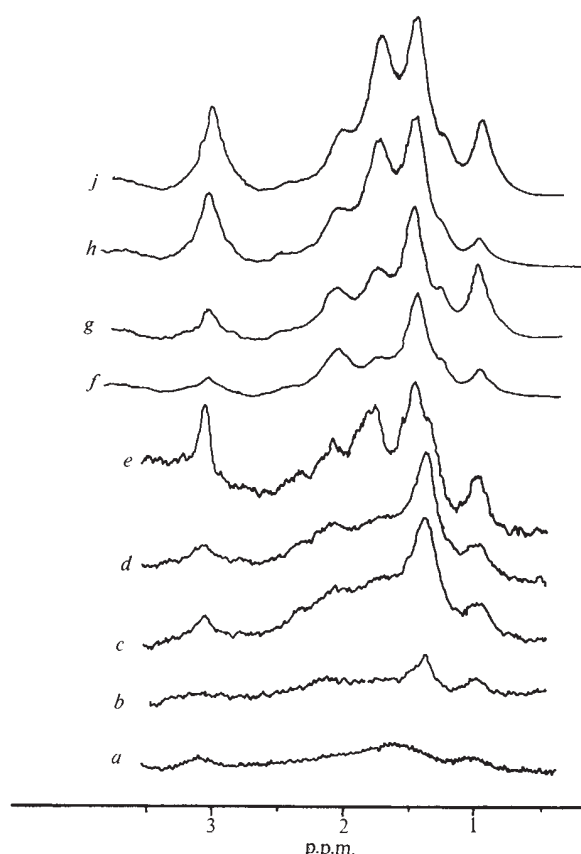


Fig. 2 High-field parts of (a). 100 MHz NMR spectrum of F1-depleted DNP; and 220 MHz spectra of total DNP in (b) D_2O , (c) 0.1 M NaCl, (d) 0.25 M NaCl and (e) 0.5 M NaCl; simulated 220 MHz spectra of histone fraction F1, (j) in random-coil form, (h) with signals from residues 47–106 broadened, (f) and (g) as (h) and (j) but with the resonances of 40 lysine residues removed.

M NaCl. No corresponding change in turbidity is shown by F1-depleted DNP (Fig. 5).

Detailed studies³ have clearly shown that in 0.5 M NaCl the NMR spectrum of F1 indicates a motion-reducing interaction involving only the section of the molecule between residues 47 and 106. This section comprises 27% of the whole molecule, but contains 75% of the apolar (Val, Leu, Ile) residues and only 14% of the total lysine residues. A model for the interaction can be formulated, based on a linear association of antiparallel molecules (Fig. 7 of ref. 3).

The 220 MHz spectra of DNP gel in the presence of varying concentrations of sodium chloride are shown in Fig. 2 b–e. The almost complete absence of signal in 2 b indicates that in the non-condensed gel in D_2O , the molecular structure is very rigid so that the spectrum is unobservably broad. Changing the ionic strength to 0.1 M NaCl (2 c) causes the appearance of peaks whose chemical shifts correspond mainly to those of alanine, proline and

lysine residues, accompanied by gel condensation. Neither the spectrum nor the condensation is found in DNP whose F1 has been removed (Fig. 2 a). Further increase to 0.25 M NaCl (3 d) causes no observable change in either the condensation or the NMR spectrum, but upon reaching 0.5 M NaCl, which produces re-expansion of the gel and corresponds to the removal of F1 from the complex, new peaks appear in the spectrum 2 e. These have the chemical shift of lysine resonances, and the resultant spectrum is identical with that of free F1 in 0.5 M NaCl, the only difference being that all the lines are broader by a factor of about 1.8. The greater linewidth found in the gel is ascribed to an increased viscosity effect within the gel structure.

A set of computer-generated model spectra are included in Fig. 2 for comparison. Fig. 2 j is the simulated spectrum of F1 in D_2O , and Fig. 2 h represents the spectrum obtained when the segment from residues 47 to 106 is assumed broadened by interaction. 2 g and 2 f are as 2 j and 2 h but with the added assumption that the signals of 40 of the 60 lysines of the molecule are broadened by interaction. These F1 spectra with lysine resonances removed correspond closely to those obtained from DNP in its contracted state. We therefore draw the following conclusions from the spectra of Fig. 2:

(a) In the salt conditions used, no spectrum appears which is not directly attributable to the molecules of F1 in the complex. No spectrum appears in samples from which F1 has been removed. This implies that up to 0.5 M NaCl, all the other components of the system, the other histone fractions, other proteins, and DNA, form a complex which is too rigid to give a high-resolution NMR spectrum. It may also be assumed that the spectrum of parts of F1 only becomes visible when those parts are not bound to the rigid complex. (b) Under conditions of low ionic strength, where there is no gel condensation, all the histone F1 is firmly bound to the complex. (c) Under conditions corresponding to gel condensation, most of the lysines of histone F1 remain firmly bound, and the spectrum which appears

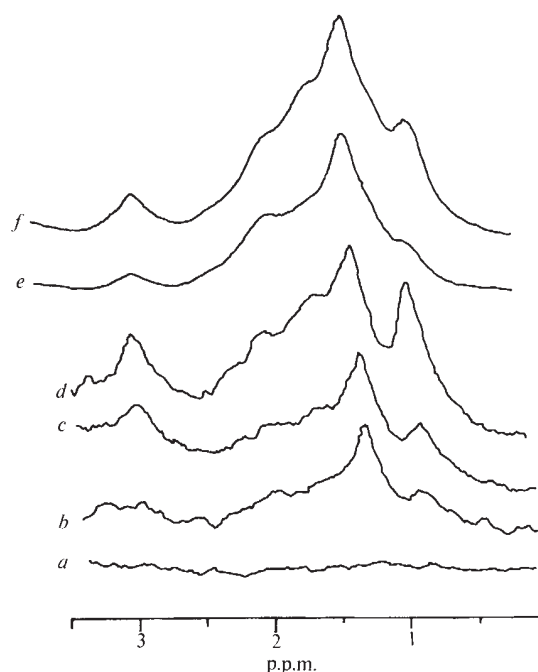


Fig. 3 High-field parts of 100 MHz n.m.r. spectra of total calf thymus DNP in (a) D_2O ; (b) 0.5 M urea- d_6 ; (c) 3 M urea- d_6 ; (d) 6 M urea- d_6 . Simulated spectra of histone fraction F1 with 40 lysine resonances removed; (e) with signals from residues 47–106 broadened and (f) in the random coil form.

Table 1 Shrinkage of Nucleoprotein Gel *

Medium	Contracted volume as percentage of initial value		
Sodium chloride	0.1 M	0.25 M	0.6 M
	18%	13%	No observable shrinkage
Magnesium chloride	0.0005 M	0.1 M	
	12%	64%	
Urea	6.0 M		
	24% expansion		

* Initial concentration of DNA in gel 6.25 mg ml.⁻¹.

is otherwise that of F1 molecules in which an interaction is taking place between segments 47-106 (compare spectra 2 c and 2 f). (d) Complete removal of the histone F1 results in re-expansion of the gel and a freeing of the lysine residues from the complex. The spectrum obtained is that of F1 with segments 47-106 interacted.

Fig. 3 shows the changes induced in the 100 MHz spectrum of DNP by dialysis against urea, and some simulated spectra drawn from the same data as Fig. 2 f and g but corrected to 100 MHz. The following points may be made about these spectra; (a) Even at 6 M urea, not all the expected lysine resonances have appeared, indicating that the F1 is still partly bound to the complex. As lysine residues would be expected to form ionic linkages, this is perhaps not surprising. (b) The peak at approximately 1 p.p.m., arising from sidechain CH_3 resonances of apolar residues, is very pronounced in 6 M urea solution, indicating that these residues, which form a major part of the interacting segment 47-106 of the F1, are very free moving and not involved in any interaction. This is very interesting in view of the fact that urea does not cause a contraction, but rather an expansion, of the gel. Urea tends to destroy hydrogen bonds and hydrophobic interactions, having far less effect on ionic linkages. Thus while there is strong evidence that histone F1 is bound to the DNP complex by ionic linkages through its many lysine residues, it also appears that active

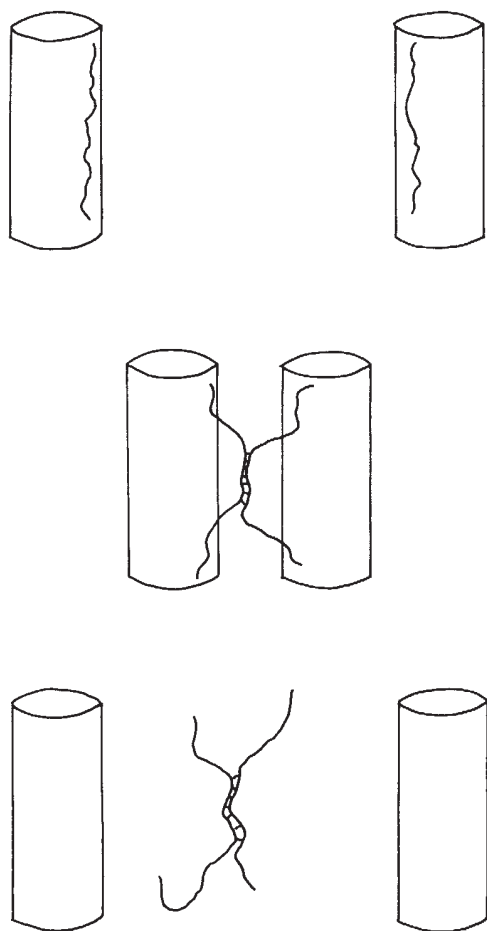


Fig. 4 Proposed mechanism for shrinkage of DNP gel. The cylinders represent all of the DNP except F1, the single lines F1 molecules. a, In H_2O , with gel expanded and F1 bound to the rest of the complex. b, In 0.1-0.3 M NaCl, region 76-106 of F1 freed from complex. F1-F1 antiparallel interactions cause gel shrinkage, as lysine-rich ends of F1 are still bound to the complex. c, In 0.5 M NaCl. The F1 is now freed completely from the complex, leaving it free to expand to its original volume.

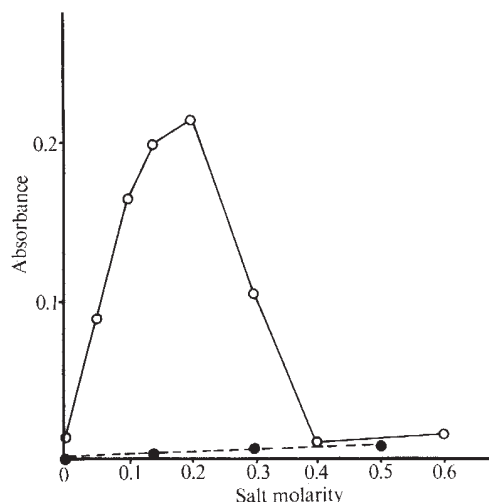


Fig. 5 Turbidities of total (DNP) and F1-depleted ($\text{P}_{0.6+\text{CMC}}$) DNP showing variation with NaCl concentration. ○—○, DNP; ●—●, Partial DNP (F1 depleted). Turbidities measured at 600 nm, 10 mm path length cells.

condensation cannot take place under conditions unfavourable to hydrogen bonds and hydrophobic interactions; interactions of these types are the most numerous in the proposed antiparallel F1-F1 interactions.

We conclude from our results that the lysine-rich histone F1 is necessary to the condensation of calf thymus DNP, and that F1 is bound by its lysine residues, which are mostly located at the amino end and in the carboxyl half of the molecule, to the rest of the DNP complex. The rest of the complex has a rigid structure which it maintains even under conditions which completely remove the F1. Condensation of the gel is associated with the freeing of large parts of F1 from the complex, while retaining some binding *via* the lysine residues, and the subsequent setting up of an interaction between F1 molecules which involves the segments from residues 47-106. The breaking of the ionic bonds holding the F1 to the rest of the complex coincides with complete re-expansion of the gel. A proposed mechanism for the contraction of DNP gel incorporating these conclusions is given in Fig. 4.

There have been several suggestions that modifications of histones are involved in controlling the physical state of chromatin¹⁴. Langan¹⁵ has clearly demonstrated the phosphorylation of serine residues in rat liver F1 and suggested that such modifications might change the F1-DNA interactions. In this respect Cole (private communication) has suggested that phosphorylation of the serines (37 and 106) at each end of the interacting segment may control changes in the interactions of the central segments. This, together with the observations that phosphorylation of the very lysine-rich histone occurs at the time of chromosome condensation, leads to the interesting speculation that phosphorylation, rather than changes in ionic strength, may serve as the trigger to condensation processes similar to those described above in living systems.

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Nuclear DNA Contents of Coelacanth Erythrocytes

AMONG the living fishes, groups of widely different phylogenetic antiquity may be classified by equally diverse sets of characteristics, both morphometric and biochemical. One such character that has been used in the study of the relationships between the major groups is cellular DNA content. It is now well known that, among fishes, modern teleosts have the lowest diploid levels (as low as 0.8 pg) and that elasmobranchs have somewhat intermediate levels (3–15 pg). The Dipnoi have the highest levels among living vertebrates (160–285 pg), and these values are approached most closely by some (but by no means all) Amphibia, such as *Necturus* and *Amphiuma* (205 and 192 pg respectively)^{1,2}.

Table 1 DNA-Feulgen Content of Erythrocyte Nuclei

Species	Number of nuclei measured	Mean Feulgen dye content in arbitrary photometric units $\pm$ error of the mean	DNA content per diploid genome ($\times 10^{-12}$)
<i>Xenopus laevis</i>	10	47.5 $\pm$ 2.2	6.3
<i>Rana pipiens</i>	10	115.7 $\pm$ 2.8	15.3
<i>Latimeria chalumnae</i>	20	99.7 $\pm$ 1.7	13.2

Fresh blood was smeared on microscope slides and allowed to dry. Slides from all three species were fixed and stained as a single batch. The preparations were post-fixed in ethanol-acetic acid (3:1) for 3 min, hydrated, hydrolysed in 5 N HCl for 1 h at 25° C and stained for 1 h with the Feulgen reagent. Dye contents of individual nuclei were measured by the two wavelength method of microspectrophotometry ($\lambda^1=560$ nm; $\lambda^2=487$ nm). The relative dye contents are given in arbitrary photometric units. The absolute DNA contents for *Rana* and *Latimeria* were estimated on an assumed value of 6.3 pg per diploid genome for *Xenopus*³. The estimated value for *Rana* agreed with literature values⁶.

In view of this great diversity of nuclear DNA content in fishes, and indeed all vertebrates, it has not always been clear what level might be considered "primitive" and what "advanced"³. It would therefore be extremely interesting to know the nuclear DNA content of supposedly primitive organisms. We have studied blood samples of a live coelacanth *Latimeria chalumnae* Smith, captured by a recent American-British-French expedition to the Comore Islands⁴. Cytophotometric measurements of Feulgen-stained erythrocyte nuclei show that the DNA content of *Latimeria* erythrocyte nuclei is 13.2 pg (Table 1). (This confirms predictions about histological thin

sections of coelacanth tissues^{2,7}.) Coelacanths therefore have diploid DNA levels well above some Amphibia, such as *Xenopus* (6.3 pg per diploid cell³) and in the same range as *Rana* (15.3 pg, Table 1) and elasmobranch fishes such as *Squalus acanthias*² (14.7 pg).

The DNA content in *Latimeria* was higher than would be expected in the ancestor of all tetrapods and we conclude that in the course of evolution from the original dipnoan-cross-opterygian-amphibian stock, increase in the coelacanth genome has occurred. Thus increased cellular DNA contents have arisen many times, independently, in this whole assemblage.

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Trans- β -farnesene, Alarm Pheromone of the Green Peach Aphid, *Myzus persicae* (Sulzer)

THE chemical structure of the repellent odour found in the cornicle secretion of the aphid *M. persicae*¹ has been identified as trans- β -farnesene.

We used two different methods to obtain material for gas chromatographic and mass spectrum analysis. In the first, a stream of dry nitrogen was passed over a homogenate of 0.6 g of aphids into a cold trap (-60° C). The collected material, dissolved in pentane, gave a positive bioassay by repelling other *M. persicae* on a radish plant. Gas chromatography (g.c.) at 115° C on an 'OV-17' column gave a single peak eluting at 7.5 min. After elution of this peak a 3° min⁻¹ programme to 190° C caused the elution of a large number of peaks beginning at 175° C. A g.c. mass spectrum of the 7.5 min peak gave a molecular ion of $204\ m\ e^{-1}$ and three prominent ions in decreasing order of 69, 93, and $41\ m\ e^{-1}$. Other characteristic ions were 120, 133, 161, and $189\ m\ e^{-1}$.

A second sample was prepared by soaking 2 ml. of *M. persicae* in 2 ml. of refrigerated pentane for 5 days, as separate observations showed that pentane caused cornicles to open and expel fluid. A single peak eluting at 6.5 min from a 4 foot, 15% stabilized 'DEGS' column at 100° C was passed over *M. persicae* feeding on radish. The aphids responded by withdrawal of stylets from the leaf and by walking from the leaf. An aliquot (1/10) of the extract was purified by cold-trapping the 6.5 min peak from this column, and this also gave a positive bioassay. The hydrocarbon nature of the compound was verified by co-chromatography with C_{16} and C_{14} saturated hydrocarbons on a 6 foot 'UC-W98' (10%) column at 150° C. The active peak eluted shortly after C_{14} and by interpolation of elution times had a boiling point of 258° C. A g.c.-mass spectrum of the active peak prepared in this manner also gave a molecular ion of $204\ m\ e^{-1}$ with prominent ions of 69, 93, and $41\ m\ e^{-1}$ and the characteristic ions 120, 133, 161 and $189\ m\ e^{-1}$.

The mass spectra in both instances were nearly identical to that reported for trans- β -farnesene². Synthesis and mass spectroscopy of trans- β -farnesene revealed that this compound and the aphid odour were identical. Bioassay of trans- β -farnesene was carried out by crushing a sealed glass ampoule containing approximately 10 mg of synthetic trans- β -farnesene in the presence of *M. persicae* on turnip leaves. A large number responded by falling or walking from the plant.

Combined with insecticidal sprays for the control of aphids, the alarm pheromone could enhance their effectiveness. The pheromone will cause those aphids avoiding a direct hit by a spray droplet to walk over the plant and contact the insecticide.

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Note added in proof. At the time this manuscript was submitted for publication Dr W. S. Bowers, USDA, Beltsville, Maryland, informed us that he had established that trans- β -farnesene was also the alarm pheromone for the rose aphid, *Macrosiphum rosae*.

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Transplantation of Killer Endosymbionts in Paramecium

SEVERAL syngens or breeding groups of *Paramecium aurelia* contain endosymbionts which may result in the development of the killer trait¹. The symbionts are of different morphological types and it has been suggested that these are non-randomly distributed between syngens². Experiments involving crosses between paramecia with and without symbionts showed that each endosymbiont required a specific nuclear gene for its maintenance. A single gene controlled the presence or absence of endosymbionts as shown by segregation in the F₂ generation. The pattern of loss of symbionts in some of those F₂ clones varies, generally extending over a number of asexual fissions which for different endosymbionts can be from two to over sixty. Infection experiments also demonstrate the requirement for a specific gene. Infection of endosymbionts from homogenates or purified preparations occurs *via* the medium but only into particular cells possessing a specific gene^{1,3}. Finally, some endosymbionts growing *in vitro* retain their infectivity into certain paramecia (author's laboratory).

As intersyngenic matings generally do not produce viable progeny, it has not been possible to test for the presence of the maintenance gene in different syngens. Also infection of symbionts from the growth medium back into the source paramecia is not always possible. We tried to transfer endosymbionts between syngens by microinjection. Such intersyngenic transfer, of kappa endosymbionts between syngen 2 and 4 have been

recorded⁴. Experiments of this sort may tell us about the distribution of the maintenance genes within the syngens of paramecia, thereby indicating genetical and geographical relationships between symbionts and host paramecia.

Paramecia were grown in grass medium. The transplantation or microinjection technique involves transferring cytoplasmic material from one cell to another with a Fonbrunne micromanipulator (ref. 4 and J. Knowles, personal communication). The presence of endosymbionts was scored by the method of Beale and Jurand⁵ or by squashing cells and examining exudates under the phase contrast microscope.

Table 1 Successful Transfer of Symbionts

Symbiont injected†	Recipient cells* which accept symbionts			
1(540) mu‡	1-540(25)§	1-551(39)	1-90(18)	8-138(14)
2(7) kappa	2-7(23)	2-511(54)	4-51(7)	
2(562) alpha	2-562(31)	2-511(18)		
4(51) kappa	4-51(28)	4-116(22)	2-7(3)	
4(A ₁) kappa	4-A ₁ (15)	4-51(23)		
8(138) mu	8-138(21)	8-131(26)	1-540(5)	
8(299) lambda	8-299(8)	8-131(18)		

* None had symbionts.

† Symbionts isolated from packed cells. Paramecia were resuspended in 3 volumes of buffer (0.25 M sucrose, 100 mg ml.⁻¹ bovine serum albumin, 0.001 M potassium phosphate, pH=7.0) at 4° C and homogenized in a 'Waring' blender. After centrifugation at 2,000g for 20 min the supernatant contained the symbionts—mu 540, mu 138⁶, alpha 562 and kappa (A₁), (51)^{6,7} and lambda 299⁸.

‡ Numbers refer to the stock and syngen of *Paramecium*, for example, 1(540) is stock 540 of syngen 1.

§ Actual number of cells injected successfully. Where the symbionts could be injected 100% success was achieved.

Symbionts (kappa, mu, alpha, lambda) were prepared from mass cultures of cells^{3,6-8}, and transferred to other paramecia. At least twenty cells were injected in each experiment and there was at least 50% survival. The results of various intrasyngenic and intersyngenic transfers of symbionts are shown in Table 1.

Each endosymbiont can be successfully injected into at least one other stock within the same syngen. Successful intersyngenic transfers were observed for mu from stock 138 of syngen 8 to stock 540 syngen 1 (and vice versa), and kappa (51) of syngen 4 to syngen 2. However, mu symbionts did not grow in syngens 2 and 4, nor did kappa in syngens 1 and 8. In all cases control injections were carried out using homogenates or other cell fractions including mitochondrial and post ribosomal fractions from paramecia without endosymbionts. Post-ribosomal fractions from cells with endosymbionts were also unsuccessful in bringing about the establishment of endosymbionts. The possibility that a gene for maintenance is being transferred during microinjection has been ruled out by the following experiment.

Cytoplasm from paramecia without symbionts but which possess the gene was injected into some cells lacking the gene, for example stock 299 into stock 138. Microinjection of stock 299 lambda symbionts into the stock 138 survivors never resulted in the establishment of endosymbionts. Incidentally, successful microinjection of endosymbionts into paramecia which previously contained the same symbionts has been recorded (Table 3). Several experiments have been carried out where endosymbionts were transferred from one paramecium to another with different endosymbionts (Table 2).

The maintenance of a particular endosymbiont depends on environmental conditions and on the presence or absence of a nuclear gene. Successful injection implies the presence of a specific symbiont-supporting gene in the recipient. Cells which do not grow the symbionts following injection would then lack the gene. The exchange of kappa particles between syngens 2 and 4 and mu between 1 and 8 would mean that the gene in the recipient cell can function with close relatives of the resident endosymbionts or else that there is a second gene in the recipient similar to that of the donor cell. I therefore examined the effect of the nuclear gene on symbiont maintenance. Different symbionts were injected into stocks which have recently lost the

maintenance gene as determined by breeding experiments and results are shown in Table 3.

It is clear that in some cases endosymbionts can be transferred to cells without the gene and that they persist through many (>100) fissions. These cells were tested by mating to paramecia with the endosymbionts and were shown to lack the gene, as judged by the appearance of 1:1 segregation of clones which maintain or lose endosymbionts in the F_2 generation. Evidence for the gene which does not allow maintenance of mu 540 in stock 513 has been recorded elsewhere⁹, and the presence of a gene which does not allow alpha maintenance (here in stock 576) has also been recorded².

The presence of the gene which could not maintain kappa 51 was shown by making a cross between stock 51 (with kappa) and stock 116. Following autogamy of the hybrid, an F_2 was obtained comprising 25 clones capable of maintaining kappa and 30 unable to do so, indicating a 1:1 ratio as would be expected for segregation of a pair of alleles. In some experiments there was no gene difference between stocks 131 and 138 in terms of maintenance of mu 138¹. By contrast, I have found a gene difference between the two stocks. Stock 131 also possesses a gene which does not allow lambda 299 to grow in the cytoplasm.

The most surprising result, however, is that with the exception of mu 138 and mu 540 which seem to be interchangeable, the resident symbionts only can be maintained in cells which lack the specific maintenance gene. For example, it is possible to transfer mu 540, lambda 299 or alpha 562 to paramecia which have recently lost the same endosymbiont following replacement of the particular maintenance gene during breed-

ing. This result is similar to the long term maintenance of endosymbionts in the absence of the gene as described elsewhere¹⁰.

However, successful injection of alpha, lambda, and mu only takes place in such cells if they are not "older" than twenty, thirty and twenty fissions respectively following gene replacement. Cells at later fissions following replacement of the gene do not accept the endosymbiont which was previously present. This result suggests either that the genes for maintenance are still active for a certain period, then become inactive or that genes which prevent the growth of symbionts become active after a delay. The maintenance gene in the latter case is inactive and the alternative allele produces an inhibitor which functions to prevent the survival of the endosymbionts. To explain the infection of stocks 131 and 116 which will possess this active gene and also the fact that successful injections can only occur over twenty fissions, we must also postulate that the gene is only active under certain conditions.

In summary, symbionts can be microinjected successfully between paramecia independently of the presence or absence of the major gene, stock and even syngen. Such results may seem to suggest that the growth and replication of the endosymbionts do not depend on specific genetic factors or, alternatively, that a similar gene is present in different syngens and protozoa.

Symbionts have been introduced into another protozoan, *Didinium*^{10,11}. The symbionts can also grow *in vitro* in the absence of the genes with a similar growth rate as *in vivo*, showing that the locus does not control the growth rate¹². Opposite conclusions were reached in infection experiments

Table 2 Injection of Symbionts into Paramecia with Other Symbionts Present

Recipient cells*	Symbionts injected						
	Mu (540)	Mu (138)	Lambda (299)	Kappa (51)	Alpha (562)	Kappa 7	Kappa A ₁
Mu 540	— (10)†	✓ (9)	✓ (31)	✓ (26)	✓ (20)	✓ (9)	✓ (15)
Mu 138	✓ (15)	— (7)	0 (15)	✓ (18)	✓ (6)	X (12)	✓ (9)
Kappa 51	✓ (8)	✓ (15)	✓ (6)	— (9)	✓ (8)	✓ (7)	✓ (10)
Lambda 299	✓ (21)	✓ (18)	— (2)	✓ (8)	✓ (5)	X (15)	X (11)
Alpha 562	✓ (10)	✓ (21)	✓ (15)	✓ (15)	— (19)	✓ (8)	✓ (12)

— = Impossible to tell if maintained as same symbiont injected. However, injection carried out as a control and symbionts were maintained in all cases.

✓ ✓ = Both symbionts retained.

✓ = Resident symbiont only retained.

X = Injected symbiont only retained.

0 = Neither particle retained.

* At least twenty cells were injected and the results represent all the survivors in each injection.

† Actual number of survivors which maintain or reject injected symbiont.

Table 3 Injection of Symbionts into Paramecia which Have Recently Lost Symbionts

Recipient cells*	Symbionts injected						
	Mu (540)	Mu (138)	Lambda (299)	Kappa (51)	Alpha (562)	Kappa 7	Kappa A ₁
540	✓ (25)†	✓ (29)	0 (34)	0 (32)	0 (17)	0 (16)	0 (15)
513							
138	✓ (29)	✓ (18)	0 (29)	0 (18)	0 (19)	0 (15)	0 (11)
131							
51	0 (10)	0 (17)	0 (7)	✓ (17)	0 (25)	0 (13)	0 (8)
116							
299	0 (15)	0 (15)	✓ (19)	0 (16)	0 (23)	0 (12)	0 (17)
131							
562	0 (30)	0 (20)	0 (15)	0 (20)	✓ (15)	0 (9)	0 (4)
576							

* Cells which lost symbionts in the 20 asexual fissions before injection. These were produced by mating cells with endosymbionts to those without and passing F_1 clones through autogamy. Where one gene is involved in maintaining the symbionts this breeding programme results in clones of cells which lose their symbionts. At least 20 cells were injected.

† Number of cells injected which survive and which show maintenance or absence of symbionts.

✓ = Symbiont injected in 100% of cases.

0 = Symbiont rejected in 100% of cases.

and genetic experiments where a specific gene was deemed to be necessary for the growth and reproduction of the symbiont^{1,7}.

My results are consistent with the presence of a genetic locus which is involved with the maintenance of endosymbionts. It is not clear, however, if the activity of the locus involves, (1) protecting the symbiont from destruction during the early fissions after conjugation or infection, or (2) producing a destructive agent which prevents the maintenance of the symbionts. Also the results presented here make it necessary to postulate that the gene may not always be active. The apparent specificity of a locus for one endosymbiont is still consistent with either mechanism.

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Cyclophosphamide: Induction of Superovulation in Rats

THE number of ova released at ovulation in rats varies within a narrow limit, usually between 10 and 15. Factors which maintain this relative constancy are not known. Several agents capable of inhibiting ovulation are known^{1,2}, but apart from gonadotrophin injections there appears to be no way to increase the normal ovulation rate⁷. An agent capable of doing this might be useful in improving breeding potential of laboratory or farm animals, and provide a tool for the study of the neuroendocrine basis of superovulation. (Superovulation is defined as an increase in the ovulation rate beyond that found in the controls.) Exposure of mice³, rats⁴ and hamsters⁵ to X-rays has been found to increase ovulation rate and/or litter size. As radiomimetic agents⁶ duplicate several effects of X-rays, they might be expected to induce superovulation in rats. Only cyclophosphamide (CPA) amongst those we tested had this effect.

Adult sexually mature rats (Wistar strain) were maintained under controlled environmental conditions of light (from 0600 h to 2000 h) and temperature (22°C). Vaginal smears were taken daily and rats which had at least one and frequently more consecutive 4 day oestrous cycles were included in the study. One group of five rats received a single oral dose of CPA (50 mg kg⁻¹) in 0.2 ml. of 0.5% 'Tween 80' in saline, the other received vehicle alone. The progression of oestrous cycles following CPA treatment remained unaffected and all treated animals showed cornified smears as in controls on the expected day. When rats were killed on this day, about 100 h after CPA administration, the number of tubal ova recovered from the CPA treated rats was significantly higher than from the control rats (Table 1). The uterine and ovarian weights of

the treated rats were comparable to those of controls (Table 1). Except for a slight loss in the body weight (4.2%) no other side effects of the treatment were observed. When this experiment was repeated, the mean ovulation rate in the treated rats was 17.2 ± 1.3 ($n=5$) which was again significantly higher than that in the control rats (10.5 ± 0.5 ; $n=23$). When the drug was administered on other days of the cycle, metoestrus, dioestrus or pro-oestrus, it failed to increase ovulation rate and the length of the treated cycle remained unaffected. Increasing the dose to 100 mg kg⁻¹ also failed to cause any further increase in the number of ova shed. Histological examination of serial sections of ovaries of rats treated with CPA and killed on the morning of oestrus showed increased numbers of freshly formed corpora lutea as expected. No luteinized follicles with entrapped ova could be found.

Table 1 Effects of Oral Administration of CPA on Ovulation Rate in Rats

Group	n	Ovulation rate	Ovarian wt (mg)	Uterine wt (mg)
Control	5	12.2 ± 0.37 (11-13)	73.3 ± 5.7	519 ± 33
CPA treated	5	$17.6 \pm 1.29^*$ (15-22)	81.7 ± 3.0	457 ± 34

* $P < 0.01$ when compared with control.

CPA (50 mg kg⁻¹) administered on day of oestrus. All animals had tubal ova. Values, mean $\pm$ s.e. Figures in parentheses denote range.

To determine whether ova shed in the CPA treated rats were physiologically normal and capable of fertilization and implantation, the experiment was repeated but females were housed with fertile males on the night of first pro-oestrus following CPA treatment. Mating was confirmed on the following morning by finding spermatozoa in the vaginal smear (day 1 of pregnancy). At autopsy on the ninth or fourteenth day of pregnancy, the numbers of implantation sites and corpora lutea were found to be significantly higher than in controls (Table 2). A gross examination of embryos revealed no abnormalities. Embryo loss, that is the disparity between the number of corpora lutea and implantation sites, was significant in the CPA treated rats ($P < 0.05$) but not in the control animals, and it seemed to increase with advancing gestation. This may be due to crowding of embryos in the uterus with an increase in competition for nutrients. Increased embryo mortality is also known to occur in animals superovulated with gonadotrophin treatment⁷.

Table 2 Effects of CPA on Number of Corpora Lutea and Implantation Sites in Rats

Parameter	Autopsy on day 9		Autopsy on day 14	
	Control	Treated	Control	Treated
No. of rats	9	9	15	10
No. corpora lutea	13.1 ± 0.7	$18.4 \pm 1.0^*$	12.2 ± 0.4	$18.6 \pm 1.4^*$
No. implantation sites	$12.0 \pm 0.4^\dagger$	$16.7 \pm 1.0^\ddagger$	$11.4 \pm 0.4^\dagger$	$15.0 \pm 1.0^\ddagger$

* $P < 0.01$ when compared with the contemporary control group.

† Not significantly different ($P > 0.05$) from the number of corpora lutea in the same group.

‡ Significantly different from the number of corpora lutea in the same group.

Oral administration of CPA, 50 mg kg⁻¹. Values, mean $\pm$ s.e.

The data show that a single oral administration of CPA at an appropriate stage of the oestrous cycle markedly increases ovulation rate. This may result either from an increased gonadotrophin secretion from the pituitary, permitting maturation of increased number of follicles, and/or from decreased rate of atresia of ovarian follicles. We believe this is the first report of a chemical substance unrelated to gonadotrophins capable of increasing ovulation rate.

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Effect of Multiparity on Human Maternal Hypersensitivity to Foetal Antigen

ALTHOUGH the infant possesses histocompatibility antigens which differ significantly from those of the mother, the foetal allograft manages to survive the period of gestation without being rejected. To explain this apparent paradox of transplantation immunology, Medawar in 1953 advanced several hypotheses¹. These included (1) that the uterus may be an immunologically privileged site; (2) that the foetal antigens are weakly expressed; (3) that there may be an anatomical barrier separating the maternal and foetal circulations, and (4) that pregnancy may be accompanied by some reduction in the immunological capacity of the mother. We have attempted to evaluate one aspect of this problem using an *in vitro* correlate of cellular hypersensitivity to study the immunological response of the mother's lymphocytes to those of her infant. This technique, the macrophage migration inhibition assay², measures the elaboration of a soluble material, migration inhibitory factor (MIF), from sensitized lymphocytes after stimulation by specific antigen^{3,4}. Previous work with mice has shown that when peritoneal exudate cells from two genetically different strains were mixed together for 24 h their migration was not inhibited, that is, no MIF was generated, unless one strain had been previously sensitized to the other⁵. In these conditions, the assay appeared to be measuring a cellular immune response indicating prior sensitization. This is in

contrast to the response observed in the standard mixed lymphocyte reaction which measures at 5 days the incorporation of ³H-thymidine into lymphocyte DNA. The latter response records an event of primary sensitization between lymphocytes of individuals with different histocompatibility antigens.

Table 1 lists the clinical data from the mothers and infants studied. Normal unrelated individuals with no history of transfusions served as controls. In addition, two patients with histories of multiple transfusions and one patient who had received a kidney graft were also studied.

The method for the production of MIF by human peripheral lymphocytes has been described previously⁶. Briefly, heparinized blood was sedimented, and the lymphocytes were separated from the monocytes and polymorphonuclear leucocytes by means of cotton columns. The lymphocytes were washed and the cell count adjusted to 3×10^6 lymphocytes/ml. Cells from each donor were cultured separately in medium TC-199 (free of serum or antibiotics) as well as being mixed with cells from other individuals in a 5:1 ratio (responder:stimulator). Cell-free supernatants were harvested daily for 3 days, concentrated and assayed for MIF activity on normal guinea-pig macrophages in capillary tubes⁶. The migrations were drawn at 24 h, the area was determined by planimetry, and the percentage migration was calculated using the following formula

$$\% \text{ migration} = \left\{ \frac{\text{area of migration } \bar{xy}}{(\text{area of migration } \bar{x} + \text{area of migration } \bar{y})} \right\} / 2$$

where x is in supernatants from cultures of responder cells; y is in supernatants from cultures of stimulator cells; xy is in supernatants from cultures of mixtures of responder and stimulator cells. Supernatants were assayed on cells from two or three guinea-pigs, and the areas of migration from four or six capillaries were averaged.

Results of the MIF assay in cultures obtained from normal and experimental subjects are illustrated in Table 2. It can be seen that lymphocytes from control subjects with no previous history of transfusions, when mixed with lymphocytes from unrelated individuals, did not produce substances during culture that were inhibitory to guinea-pig macrophages. The mean percentage migration for the group was 101.9 ± 9.9 (s.d.). Migration less than 81%, 2 s.d. below this mean, was considered positive and indicative of MIF production. To determine whether we could detect prior sensitization, three patients, two with histories of multiple transfusions, and one, a recipient of a kidney graft, served as potential positive controls (the latter was tested with lymphocytes from the kidney donor).

Maternal lymphocytes from five out of ten patients (M. Q., D. F., V. C., M. S., M. M.) produced MIF when incubated in the presence of their infants' lymphocytes. In addition, two of these mothers were tested against lymphocytes from unrelated donors and did not produce MIF. The only distinction between mothers whose lymphocytes made MIF and those who did not was their parity (see Table 1). All five of the

Table 1 Clinical Data

Patient	Age	Parity	Transfusion history	Maternal blood type	Gestational age (weeks)	Sex	Infant birth weight (g)	Abnormality
D. R.	18	I	0	A+	41	M	3,550	Normal
R. T.	23	I	0	O+	38	M	3,020	Normal
G. G.	19	I	0	AB+	39	F	3,500	Normal
K. M.	22	II	0	A-	38	M	3,250	Coombs-Rh+
D'Ag	22	III	Multiple* 1963	B+	39	F	3,400	Normal
D. F.	29	III	0	A+	40	F	3,501	Ventricular septal defect
V. C.	21	III	0	O+	40	M	4,100	Normal
M. M.	33	III	1-1968	B+	39	M	3,300	Normal
M. S.	23	V	1-post partum	A+	38	M	4,000	Normal
M. Q.	22	VII	0	A+	18	M	400	Normal

* Received radiation therapy for carcinoma of ovary.

Table 2 Percentage Migration

Normal subjects*	Patients†	Mother-baby‡	Baby-mother‡
98	79	87	97
98	76	90	117
96	75	101	111
93		87	91
122		97	106
112		68	110
100		71	96
101		71	109
98		76	102
91		62	89
Mean $\pm$ s.e.m. 100.9 $\pm$ 2.8 76.7 $\pm$ 1.2 81.0 $\pm$ 4.0§ 102.8 $\pm$ 2.8			

MIF production in control subjects and patients. Supernatants obtained from mixed lymphocyte cultures in the absence of serum (responder: stimulator ratio being 5:1) were assayed for MIF activity. Migration below 82% is significant. All mothers whose lymphocytes produced MIF in response to their babies' lymphocytes had three or more pregnancies.

* No previous blood transfusions.

† Two patients with multiple blood transfusions and one kidney recipient.

‡ The MIF results for each mother and infant are listed in the same order that is given in Table 1.

§ Mean percentage migration with three or more pregnancies is 74.2% $\pm$ 4.9; mean percentage migration with less than three pregnancies is 91.3% $\pm$ 3.3 (difference of mean migrations statistically significant by the Student *t* test for paired means = $P < 0.05$).

mothers whose lymphocytes were sensitized to those of their infants had been pregnant three or more times. In all but one where sensitization was not detected, the mothers were in their first or second pregnancy. The one exception was a gravida three para three woman who had received X-irradiation and chemotherapy for an embryonal cell carcinoma of the ovary seven years prior to the current pregnancy. These therapeutic modalities may have altered the patient's cellular immune response. It was also interesting that when infants' lymphocytes were mixed in a 5:1 ratio with maternal lymphocytes, no MIF was made, that is, there was no evidence of sensitization of the infants' to the mothers' cells.

These data demonstrate that cellular hypersensitivity to the infant is present in some mothers during pregnancy. Therefore, the success of the foetus as an allograft is not a result of immunological paralysis as previously suggested⁷. But the question remains: what is the mechanism which allows foetal survival in these conditions? Recent studies have suggested that the maternal "tolerance" during pregnancy may be mediated through serum factors. The Hellströms, for example, have shown in mice that maternal serum contains an enhancing factor which abrogates the cytotoxic effect of maternal to foetal cells⁸. Further, Ceppellini has demonstrated a factor in maternal serum which suppresses the autologous and homologous responses in the mixed lymphocyte reaction⁹. Our studies were carried out in the absence of serum and, therefore, such factors would not mask the production of MIF. It remains to be determined whether preincubation of foetal cells with maternal serum would block the subsequent production of MIF by sensitized maternal lymphocytes.

In the studies reported here, MIF production seemed to reflect prior sensitization. Others, however, have detected an inhibitory activity in supernatants obtained from mixtures of nonsensitized cells^{10,11}. Bartfeld and Atoynatan¹⁰ required 2.5–5 times more cells than in the present study to elicit "MIF production" in mixtures of nonsensitized cells; in the experiments of Phillips *et al.*¹¹ a longer time course was also required. It is possible, therefore, that these workers may be detecting primary allograft sensitization reactions which our technique is not measuring either because of differing experimental conditions or because it is less sensitive.

In conclusion, in the experimental conditions used in this study, it has been possible to demonstrate that cellular hypersensitivity to the infant is present in some mothers during

pregnancy and, further, that the incidence of maternal lymphocyte sensitization increases with the number of pregnancies. The sensitization of the mother by foetal erythrocytes resulting in rhesus sensitization is a well known phenomenon. Exposure of the mother to foetal cellular antigens during gestation with antigens passing from the foetal to the maternal circulation may be the source of the maternal sensitization herein observed. Such crossing of the placenta by maternal and foetal antigens has been described^{12,13}.

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Bladder Cystitis following Focal Infection of Group A Streptococci

Foci of infections have long been recognized as the cause of certain systemic diseases. In 1915, Rosenow studied the localization of streptococci after i.v. injection¹. Subsequent studies have shown that viable streptococci and/or their metabolic products localize in nearly every organ and tissue²⁻⁴.

The localization of Group A streptococci in relation to the urinary bladder is of general interest. Meisser and Bumpus⁵ found bladder lesions after i.v. injection of a "streptococcus". Much interest in the field of urology has been generated over the aetiology of interstitial cystitis or Hunner's ulcer, which is almost exclusively a disease of women. Evidence from our laboratory has shed some new light on the possibility of the causative factor being a Group A streptococcus⁶. Using immunofluorescent techniques streptococcal antigens were demonstrated within the bladder wall following subcutaneous injections of lyophilized *Streptococcus pyogenes* Group A, Type 12, in the back of rabbits. Microscopic examination of these bladders showed chronic lesions characterized by inflammatory cell invasion, general tissue injury and alteration, fibrosis, bladder contraction, depletion of DNA fluorescence, and circulating anti-DNA antibodies, which showed many similarities to human interstitial cystitis. No viable streptococci

were present. Preliminary studies have shown a sex difference in these lesions with female rabbits evidencing a severe bladder lesion in contrast to males.

We report this in view of the severity of Group A streptococcal involvements in other diseases such as rheumatic fever, glomerulonephritis and their complications.

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Tryptophan Excited States and Cataracts in the Human Lens

WE present here a study of the fluorescence and phosphorescence of human eye lenses *in vitro* with regard to the mechanisms of formation of cataracts. Light transmission by the cornea decreases rapidly with decreasing wavelength, and is essentially zero for wavelengths less than 293 nm¹⁻³. The important effect of this corneal absorption was demonstrated by Bachem⁴ who induced cataracts in guinea-pigs and rabbits with ultraviolet light and presented an action spectrum to show that the efficiency of *in vivo* cataract production rose steeply, from zero at 293 nm to maximum at 300 nm, and then fell off sharply at 313 nm with a long tail extending to about 365 nm. This action spectrum and the corneal absorption spectrum are shown in Fig. 1.

We used normal lenses obtained from the Eye Bank, and cataractous lenses obtained directly from surgery. Lens specimens from donors aged 39-81 years were placed in sterile Ringer's solution, immediately in the case of cataracts from surgery, and within 45 min of death in the case of normal lenses. The solutions were then frozen to dry ice temperature, and kept until needed. Measurements were

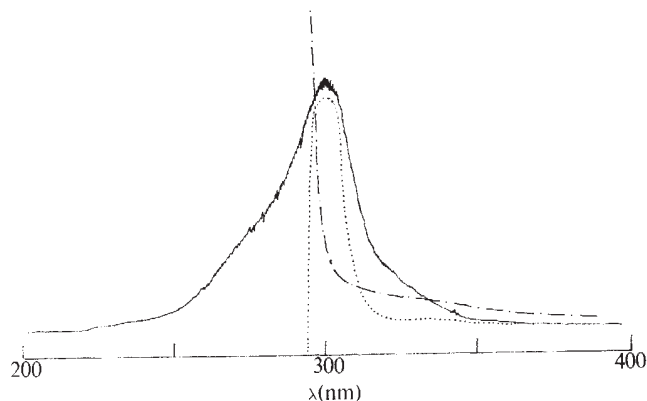


Fig. 1 Phosphorescence excitation spectrum of a healthy intact human lens at 77 K. The emission is monitored at 440 nm. —, Excitation spectrum; . . . , action spectrum of Bachem⁴; - - -, corneal absorption.

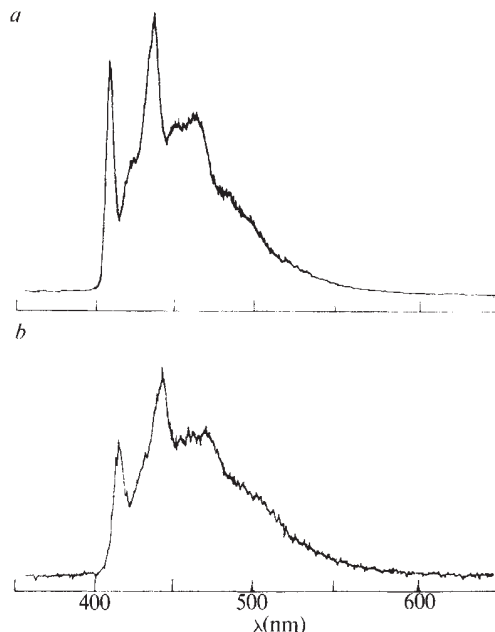


Fig. 2 a, Phosphorescence emission of tryptophan in ethanol-water solution (10^{-3} M) at 77 K, excited at 300 nm. b, Phosphorescence emission of an intact healthy lens at 77 K, excited at 300 nm.

made from within a day to within a month of enucleation. For spectroscopic emission studies, the samples were mounted in quartz cells. The excitation beam from a new 1,000 W xenon arc lamp passed through a Bausch and Lomb compact grating monochromator, producing an excitation band width of about 7.5 nm. The emission was observed at right angles through a second 'Bausch and Lomb' monochromator, and detected photoelectrically with a '1P28' phototube, the output of which was recorded on an X-Y recorder. A rotating can phosphoroscope was used to separate short lived fluorescence emission from long lived phosphorescence emission. (The spectra presented have not been corrected for lamp output or phototube wavelength dependences. When these corrections are made, the phosphorescence excitation maximum is shifted about 5 nm to the blue and the phosphorescence emission peaks are each shifted about 5 nm to the red.) Absorption spectra were measured on a 'Cary 14' spectrophotometer. Intact lenses were pressed between quartz plates to adjust the optical density for these measurements.

We have measured the phosphorescence spectra of intact human lenses at 77 K, and find a characteristic emission spectrum (Fig. 2). This spectrum is independent of exciting wavelength in the range 250-313 nm. (For excitation wavelengths longer than about 313 nm, the phosphorescence emission changes from that shown in Fig. 2 to a broad structureless spectrum with a maximum at about 520 nm.) This phosphorescence was compared with that of tryptophan and we concluded that the former originates from tryptophan residues in the interior of a protein, and not from the free amino-acid (Fig. 2). This is shown by the redshift of the peaks in the lens phosphorescence spectrum⁴ and by lens fluorescence data (ref. 5 and Staton and Borkman, unpublished data).

At 77 K, the phosphorescence of tryptophan in EPA or ethanol-water glass was found to have a lifetime of 7.0 ± 0.5 s, while that of the normal lenses was 4.6 ± 0.5 s, and that from a brunescant cataractous lens was found to be 5.2 ± 0.5 s. Decay data on large numbers of normal and cataractous lenses are needed to improve the precision of the lifetime measurements. Our data, however, clearly show that the lens emission originates from tryptophan within a protein, and not from the free amino-acid⁵. They also suggest the possible use of emission lifetimes as a new criterion for characterizing

cataracts. The change in lifetime between a normal and a cataractous lens could result either from the presence of a new phosphorescence species, or from the fact that the local environment of tryptophan⁵ may be different in normal and cataractous lenses.

Monitoring the tryptophan-like lens phosphorescence at 440 nm, we obtain an excitation spectrum peaking at 300 nm as shown in Fig. 1. After correcting for the wavelength dependence of our excitation lamp output, we find the maximum in the phosphorescence excitation spectrum to be at 295 ± 5 nm, a range which clearly overlaps the wavelength reported by Bachem³ for maximum efficiency in ultraviolet cataract production. A good approximation to Bachem's action spectrum for cataracts can in fact be obtained by computing the product of the corneal transmission spectrum and our lens phosphorescence excitation spectrum. This demonstrates the potential importance of the excited states of tryptophan as intermediates in lens photodamage. This idea is supported by the fact that tryptophan is known to be very photo-reactive, both in the free state and in proteins; it forms coloured products under ultraviolet irradiation, even at liquid nitrogen temperatures⁶.

An effort was made to identify the tryptophan excited state(s) potentially significant in lens photodamage. We reasoned that a fast photochemical process originating from the excited singlet state of tryptophan would result in fluorescence quenching, but that a fast photochemical process originating from the triplet state would result in quenching of phosphorescence. At 77 K our emission spectra show that the lens fluorescence and phosphorescence quantum yields are both large (about 20%) and about equal to each other. We conclude that neither excited state emission is strongly quenched under these conditions. However, at room temperature we observe no phosphorescence from human lenses (at least 100 times weaker than at 77 K), and therefore, under these conditions, the triplet state energy tryptophan must be dissipated by processes other than light emission; chemical reaction, energy transfer, or radiationless transitions. These considerations suggest that at room temperature tryptophan excited triplet state is the intermediate in lens photodamage. On the other hand, we (and others⁷) have observed that the fluorescence of tryptophan in ethanol-water solvent at 25° C is quenched (by a factor of 2) in an aerobic solution compared to a vacuum outgassed sample. Barenboim has claimed that the mechanism of this quenching is enhanced intersystem crossing from the excited singlet to the triplet state of tryptophan⁷ and proteins containing tryptophan can take up oxygen under ultraviolet irradiation⁸. We find that irradiation of a tryptophan solution in ethanol water solution at 25° C under aerobic conditions for 16 h with 313 nm Hg line produces visibly coloured products. The absorption spectra of these products were found to be in agreement with those of the adult human lenses we studied. That these new species were photo-oxidation products was shown by irradiation of a degassed tryptophan sample for 16 h without the formation of products, as determined by ultraviolet absorption and thin layer chromatography (TLC). These fluorescence-quenching and photochemical results suggest that the excited singlet state of tryptophan may also be important in lens photodamage and that the degradative reaction is a photo-oxidation.

Our TLC measurements showed that air photo-oxidation of tryptophan results in two products. Gomyo and Fujimaki⁹ have recently demonstrated the sensitized UV-photo-oxidation of tryptophan in lysozyme, and have identified two photoproducts as kynurenine and 3-oxykynurenine. Van Heyningen¹⁰ has isolated three fluorescent compounds from the normal human lens and has identified two of them as free kynurenine and a glucoside of 3-hydroxykynurenine. We and others¹¹ have observed that the absorption and fluorescence intensities are greater in brunescant cataracts than in normal lenses. It seems likely that this lens pathology

is attributable to an excess of tryptophan oxidation products, generated enzymatically, or, as we have shown, photochemically.

Gomyo *et al.*⁹ showed that tryptophan photo-oxidation in lysozyme caused a change in protein conformation resulting in decreased water solubility. A similar decrease in the solubility of lens protein following tryptophan photo-oxidation could induce opacity in the lens. The low solubility of brunescant lens proteins, relative to proteins in other types of cataracts, has been noted¹¹, and the increase in the insoluble protein fraction with onset of cataracts in general is well known¹².

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Indices of Discriminability

Hammerton and Altham¹ have pointed out the desirability of having a numerical measure for the discriminability of two stimuli, A and B, that does not depend upon the specific assumptions about the probability distributions of the internal events produced by A and B. Assuming that the data consist of confidence ratings for A and B, they have proposed an index C, which is equal to the difference between the average ratings for A and B divided by the difference in the ratings that represent "certainly A" and "certainly B". Unfortunately their definition assumes that at least one rating can represent "certainly A" and at least one rating can represent "certainly B". This often does not happen. For example², when observers were permitted to develop their own rating systems for visual stimuli, it was found that for stimuli consisting of 55 and 66 quanta, on the average, at the cornea, there was not a single rating that meant certainly one stimulus or certainly the other. Another way of saying this is that there was never a single trial on which the observer was certain that the stimulus was the strong one.

Their index could still be applied if one divided instead by the difference between the smallest rating used for stimulus A and the largest rating used for stimulus B. However, it is incorrect to suggest that the smallest rating used for the weaker stimulus A means "certainly A" because it may sometimes be given in

response to B. Their index is actually the difference in the mean ratings for A and B divided by the range of ratings used (minus one). This is somewhat unsatisfactory because if the mean rating and range of ratings are fixed, then C is completely determined. But if only the variances of the distributions decrease, then discriminability will increase although their index C will remain unchanged.

The desirable properties that a discriminability index should include are (1) a simple interpretation that is independent of any assumptions about the underlying distributions; (2) independence from the particular units used for the ratings; (3) ability to compare different observers doing the same task and the same observer doing different tasks; (4) the index should be a function of the parameters that are necessary to specify discriminability. It must change when discriminability changes.

The index C does not have the fourth property because it is independent of the variances of the distributions. The index d' fails the fourth condition because it only depends on the variance of one of the distributions. Also, as pointed out by Hammerton and Altham, there is a weak implication that the underlying distributions of internal events are Gaussian, although the measure could be used when this is not the case. In principle, one could avoid this difficulty by redefining d' in terms of the horizontal intercept of the ROC curve on double probability paper or by using the index d'_e which is measured along the diagonal. But for non-Gaussian distributions, the interpretation of these indices may be extremely unclear so condition (1) is not really satisfied.

I propose, therefore, a new index D , the Standard Separation

$$D_{(A,B)} = \frac{\bar{i}_{(B)} - \bar{i}_{(A)}}{\sqrt{S_{(A)} S_{(B)}}}$$

where $\bar{i}_A$ and $\bar{i}_B$ are the average ratings for A and B, and $S_{(A)}$ and $S_{(B)}$ are the standard deviations of the respective rating distributions. Random ratings would produce $D=0$ and perfect discrimination would produce an infinite D providing that the observer used only one rating (zero variance) for at least one of the stimuli. The index D satisfies conditions (1), (2) and (3) and also satisfies (4) better than the indices C or d' , since it depends on the variances of both distributions. Of course, in principle, discriminability may depend on even higher moments, but in practice D may be completely sufficient.

There is also another older index that satisfies all four conditions. That is P_C , the percentage correct in a two alternative forced choice experiment, which is perhaps closest to what is meant by discriminability. In order to measure what P_C would have been from confidence rating data, one either measures the area under the ROC curve³ or simulates the experiment². This is independent of the Gaussian assumption.

The suggested new index D is applicable in a great variety of circumstances and may be the most generally applicable measure but P_C is also a generally valid measure. Under circumstances where the distributions are approximately Gaussian, and of equal variance, D is equal to d' .

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Transition Probability and Dichotic Listening

BLOOMFIELD¹ has rejected the hypothesis that in a dichotic listening situation the selection of the attended message by the subject is based only on spatial cues, and argued that the transition probabilities between words also affects their selection. Physical cues rather than spatial ones should have been referred to because it has been demonstrated that selection can be made in the absence of spatial cues if the two messages are presented in distinctly different voices². Only the variety of the rejected hypothesis which also postulates simultaneous analysis of the unattended message is considered in the theoretical treatment of results. If attenuation of the secondary channel and selection on the basis of physical cues only are postulated³, then words presented in the secondary channel have a relatively high recognition threshold. Without contextual information potentially gained from previous words, the instruction "tap" does not have a lowered threshold and we would not expect it to be heard. Our hypothesis contradicts Bloomfield's conclusion, although it is compatible with his data. Also in the presence of unequivocal physical separation (messages presented to different ears in different voices³) listeners never reported items from the secondary message. A strong stimulus set overrides even a strong response set⁴. Changing the contextual probability within a message is not "known to result in switching to the alternative channel", as Bloomfield asserts; Treisman⁵ has reported that "subjects occasionally repeated one or two words" from the secondary message when those words were more appropriate than those presented simultaneously in the primary message. Recognition of a word presented in the secondary message is only likely when that word is appropriate to the selected message. Presentation of an improbable word in the selected message may lower the recognition thresholds for more probable words presented in other messages, but this does not necessarily affect the threshold for other contextually improbable instructions such as "tap". Giving the listener a response set towards this word might reduce this threshold.

Words presented in the secondary message were more likely to be selected and repeated when they had a high transition probability with the preceding word in the primary message, and were responded to in preference to less probable words in the primary message. This may be a demonstration of the word-frequency effect in recognition thresholds^{6,7}. Such words may gain a response as a result of "sophisticated guessing"^{7,8} or a response bias⁹. When unattended words are probable in the context of the attended message, two sources of information are available to the listener. Preceding items restrict the set of probable alternatives and may lower the recognition threshold for this set, and stimulus information is available at the time of presentation. Although this information may be non-redundant or attenuated, in conjunction with the contextual information it may still be sufficient to enable a verbal response.

Bloomfield's hypothesis states that if selection is based upon both physical and contextual cues, then the relatively low probability of the instruction, in the secondary message, to tap will not "compete" with low probability words in the selected message. Selection will be based on physical cues alone. Although this argument differs from that above, the prediction is the same. When we consider only those occasions when correct shadowing (and, theoretically, attention to the primary message) was reported, then the tapping instruction was followed only when a highly probable word was presented simultaneously in the primary message. This is not in accord with Bloomfield's hypothesis, for a contextually probable word in the primary message should lead to selection of that message, and consequently the tapping instruction should not be heard. This can be explained in the theory of shared processing capacity¹⁰. This theory would argue that words which are highly probable require less processing capacity for their identification than do words which are improbable because the latter contain more information. The amount of capacity residual from

analysis would therefore be greater in the case of highly probable words, and the difference in the amounts of processing capacity available for secondary tasks might have resulted in more efficient analysis of the word "tap" when the residual capacity was greater.

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Reply

In my original article¹, I was concerned to juxtapose two different theories of dichotic listening. One of these is that the ears constitute two channels, only one of which can be fully analysed if items arrive at both simultaneously. The other theory, which I claimed was supported by my results, was that multiple criteria are used in the selection of items for analysis from a common, rapidly decaying auditory store. One criterion, of course, could be the ear at which the item arrived.

Underwood claims that Treisman's attenuation theory² could explain my results. The main finding I reported was that under left (shadowed) channel conditions where the subjects were likely to report a contextually more probable right channel item, they failed to obey a tap instruction in a second experiment. Appropriate tapping sometimes occurred, however, when the instruction coincided with a probable left-channel item that was successfully shadowed. I agree with Underwood that the last result calls for some notion of shared processing capacity³, and I would have developed that point had space permitted. I do not agree, however, that Treisman's theory is capable of explaining why subjects did not respond to "tap" when it coincided with an unexpected item to be shadowed, although they often did tap appropriately while shadowing a high-probability word. A theory which supposes that the unshadowed channel is attenuated must predict a higher probability of tapping during the presentation of the low-probability item for shadowing.

My own view, already expressed, is that items are selected on both spatial and contextual cues, so that if context does not favour one of two coincident items selection for processing will proceed on spatial cues alone, causing the tap instruction to be ignored.

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Feature Detectors and Vernier Acuity

HUMAN subjects can achieve an accuracy in a vernier alignment task of a few seconds of arc visual angle in optimal conditions¹⁻³. The inter-cone spacing in the central fovea is several times this magnitude⁴, so this performance must be achieved through some spatial information processing in the visual system.

Theories of vernier performance have concentrated on the problem of locating the position of the retinal image of one of the lines (that is, specifying the x coordinate in Fig. 1) to the requisite accuracy. Two explanations have been suggested. The first^{2,5} depends on the intensity differences at a receptor created when the retinal image of a line (modified by the optics of the eye⁶) is given a small displacement (the illumination gradient hypothesis). The second theory⁷ assumes each receptor, instead of giving a graded signal, only signals whether or not it is illuminated. The requisite accuracy is achieved by averaging over the length of the line to establish a "mean retinal local sign".

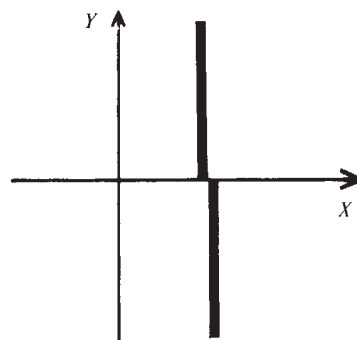


Fig. 1 The vernier acuity task. The subject makes an alignment setting by moving one of the lines along the x axis.

Neither theory is more than indirectly supported by the experimental evidence. The improvement of vernier acuity with target luminance provides some support for the illumination gradient hypothesis²; but the maintenance of acuity when the target is blurred⁸ is difficult to understand on this basis. The mean local sign hypothesis is supported by studies which find an effect of line length on acuity (ref. 7 and unpublished work of J. A. Foley-Fisher); but the sharp deterioration which is found if a separation is made between the vernier lines⁹ is not easily explained by either theory.

An alternative hypothesis is suggested by recent work on the visual system. It has been established physiologically that pattern information is transmitted by neurones in the visual cortex, often termed "feature detectors", which respond specifically to the size and orientation of elements of the stimulus array^{10,11}. The existence of similar units in the human visual system is indicated by work which demonstrates that humans possess "channels" selectively activated by stimuli of a particular size and orientation¹²⁻¹⁴. The amount of activation in a channel is determined by the contrast in the stimulus rather than the light intensity. Much current work is concerned with the elucidation of the properties of these channels and relating them to the more traditional areas of perception. Here I suggest an application of this approach to vernier acuity.

In the vernier task subjects on occasion report that they achieve their final alignment by the elimination of a "kink" at the junction of the lines. It seems possible that this might correspond to reduction of activity in a channel, as defined above, the axis of which is at an orientation oblique to the vernier lines. This is clarified in Fig. 2 which shows that the hypothesis is essentially one of contrast discrimination in such a channel. Contrast sensitivity increases as the spatial frequency

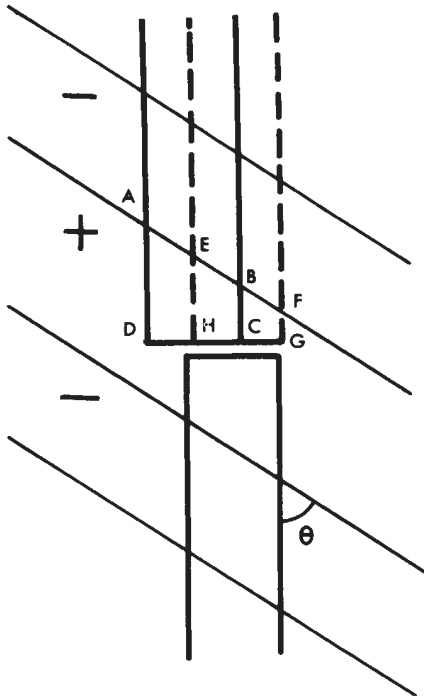


Fig. 2 The suggested hypothesis relating misalignment to activity in a cell the receptive field of which is oriented as shown at an angle θ to the vernier lines. There will be a change in excitation in the centre of the receptive field between the offset and the aligned positions, whereas the excitation of the surround will be unaffected. With the offset shown the change in excitation is equivalent to that produced by an area of target equal to the difference between the areas ABCD and EFGH. If the offset distance (DH) is d and the bar width (DC) is D , then this area is $d D \cot \theta$. This result will not be greatly altered by the transformation produced by the optics of the eye. If the receptive field width is W , the misalignment excitation change will be a function $d/W \cos \theta$ of the total produced by the stimulus. This can be regarded as equivalent to a Weber fraction for the cell and, using plausible values for the variables, fractions between 1/200 and 1/30 are obtained.

of the channel decreases (down to 8 cycles per degree)¹⁵, so it is not immediately clear whether high or medium spatial frequencies would be most effective.

The hypothesis implies that if the sensitivity of the oblique channel could be decreased, poorer acuity should result. A way of testing the hypothesis is then suggested by the method for desensitization of visual channels described by Campbell and Kulikowski¹². They show that a high contrast grating target masks gratings of a similar orientation but has negligible effect on the detection of gratings of widely different orientations. This may be interpreted to mean that presentation of a high contrast grating effectively raises the threshold in a particular channel. Thus, in order to test the hypothesis, the effect of a high contrast grating background on a vernier acuity task was determined as the angle between target and background was varied.

The vernier target was generated on an oscilloscope screen by a computer and interface ('IBM 1130, WDV'). This was viewed at a distance of 2 m in a dark room. On a second oscilloscope with identical phosphor a sinusoidal grating was generated using the technique of Campbell and Green¹⁵. A large Dove prism was placed in front of this oscilloscope and the targets were combined optically. The subject was able to adjust one line of the vernier target in either direction by means of a series of buttons interfaced to the computer which also recorded the final setting. It was found in preliminary experiments that the largest effects were obtained with medium spatial frequency backgrounds and with the vernier target of low brightness. In the main experiment the background was a 12 cycle per degree grating, with average luminance 8 cd m⁻²

and contrast about 0.6 (defined as in ref. 11). The vernier target was always vertical and was set so that when the background was also vertical, the vernier lines were just detectable.

Six members of the department, naive as to the purpose of the experiment, served as subjects; all had normal or corrected vision. The subjects made settings in ten different conditions in which the background grating was at the following angles to the vernier lines: 90°: ±45°, ±30°, ±20°, ±10°, 0°. Ten settings were made in all under each condition in two blocks of five. The sequence of the resulting twenty blocks was randomized and different for each subject. Ten minutes of practice trials preceded the experimental trials which lasted about 50 min.

The measure of acuity taken was the standard deviation of the settings about the mean. The difference between the mean and the true alignment position was also recorded, but the systematic component of misalignment was found to be generally small with no consistent trends. The data on setting variability are presented in Fig. 3 for individual subjects, and Fig. 4 shows the arithmetic means of the six subjects in each condition. Analysis of variance shows the difference between conditions is significant at the 1% level and it is also evident from the symmetry of the data that this represents a genuine angular effect. The results are in accord with the prediction that a grating background oblique to the vernier lines would impair acuity. The maximum effect occurs with a grating oriented at 20° to the vernier lines. The tuning of human orientation sensitive channels¹² is 12°–15°, and so this result is consistent with the view that maximum alignment acuity is

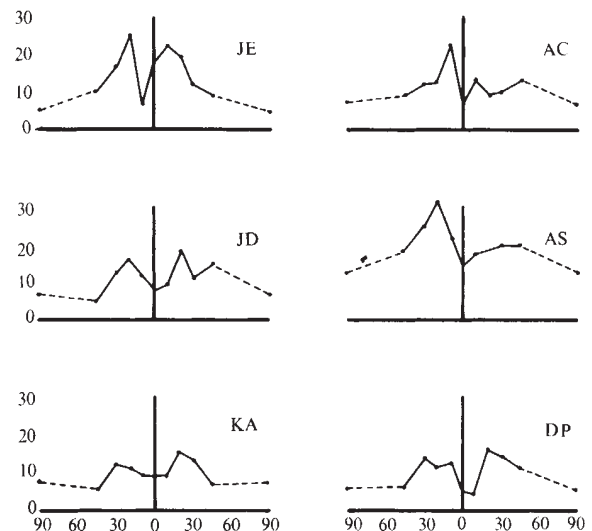


Fig. 3 Vernier acuity as a function of the orientation of a grating background. Individual results for six subjects. Each point represents the standard deviation of ten settings, measured in seconds of arc visual angle.

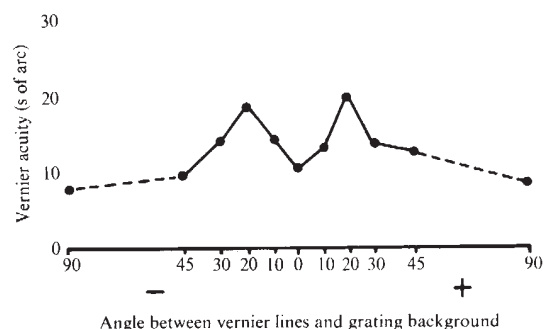


Fig. 4 Vernier acuity as a function of the orientation of a grating background. Means of the results shown in Fig. 3.

achieved by the detection of a change in the activation of an independent oblique channel.

But it is perhaps appropriate to conclude with two notes of caution. There is considerable similarity between the configuration of target and background which produces maximum acuity degradation and that which produced maximum shift in the Zollner illusion¹⁶. Thus the vernier lines in this situation are subject to a systematic illusion of tilt. It is nevertheless difficult to see why this should lead to any increase in the variance of the vernier settings, and indeed it is possible to provide an explanation of the Zollner effect in terms of feature detectors¹⁷. Finally, mention should be made of the finding of Ludvig¹⁸ that vernier precision can be maintained in a modified form of the vernier task which consists of aligning the middle one of three dots. This result presents a considerable problem for any theory of acuity.

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Effects of Marijuana and Tobacco Smoke on Human Lung Physiology

MOUSE lung explants exposed to smoke from cigarettes to which marijuana was added have been reported to display more cellular abnormalities than those exposed to smoke from cigarettes without marijuana¹. We report here a study designed to test the effects of smoke from cigarettes made of marijuana only on human lung explants, and to compare these effects with those obtained after exposure to smoke from Kentucky Standard cigarettes.

We used the model system developed for preparing and exposing lung explants to puffs of fresh smoke in standardized conditions². Fresh human adult lung tissue was obtained

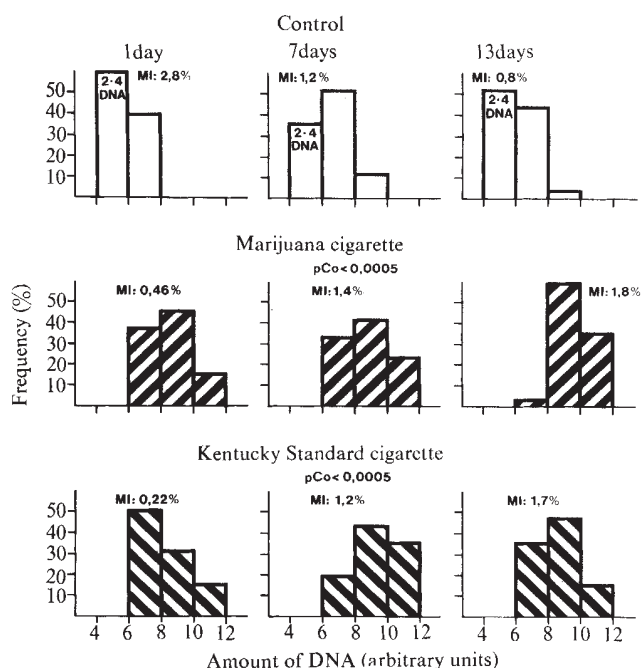
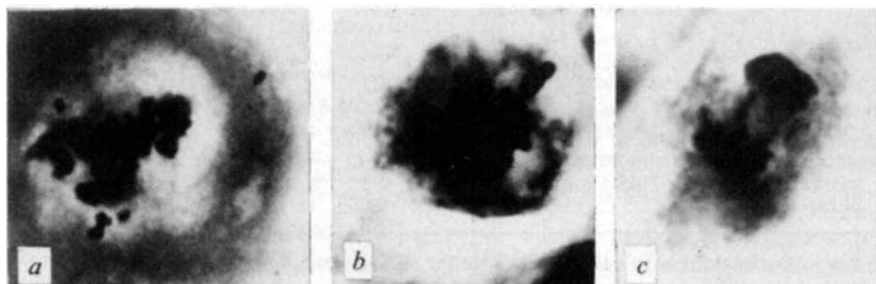


Fig. 1 Comparison between effects of fresh smoke from marijuana and Kentucky Standard cigarettes on the DNA content of fibroblastic cells in human adult lung explants, one, seven and thirteen days after exposure (Feulgen microspectrophotography). 375 cells measured. MI: Mitotic index.

from men (45–56 yr) who had undergone surgery because of pulmonary tumours. For the lung explants pieces of “normal lung” were taken far distant from the tumour. Absence of tumour was established by microscope examination. The cultures, which at three to four weeks showed a regular monolayer of fibroblastic cells, were exposed in a ‘Filtrona CSM 12’ smoking machine to puffs of fresh smoke from marijuana cigarettes, or to puffs of whole fresh smoke, or smoke of the gas-vapour phase² from Kentucky Standard cigarettes. Marijuana cigarettes containing 1.8 g of marijuana (UNC 303, 0.6% tetrahydrocannabinol (THC)) for each cigarette were prepared with the same paper as that for Kentucky Standard tobacco.

Experiments were carried out on over 1,300 cultures of human lung explants. For each experiment a minimum of eight sets consisting of twenty-four coverslips with matched lung explants was used. Each set comprised a control culture not exposed to cigarette smoke, a culture exposed to four puffs per day (25 ml. at intervals of 58 s) of fresh smoke from marijuana cigarettes for 4–10 consecutive days, and a culture exposed in the same manner to two puffs from Kentucky Standard cigarettes. The lower number of puffs from Kentucky Standard cigarettes was given because of the relatively high cytotoxic effect of this type of cigarette on the cultures. The larger puff volume of 25 ml., instead of 8 ml. used in the previous study¹, was chosen because it resembled more closely the standard puff volume of 35 ml. inhaled by human smokers. Media were changed immediately after each exposure.

Fig. 2 *a*, Metaphase of fibroblastic cell in human adult lung explant, 6 days after 4 exposures to 4 puffs of marijuana cigarette smoke (H and E, $\times 1,000$). Note tripolar metaphase with pieces of chromosomes lying distant from metaphase and the very large spindle. *b*, Metaphase of fibroblastic cell in human adult lung explant, 6 days after 4 exposures to 2 puffs of Kentucky Standard cigarette smoke (H and E, $\times 1,000$). Note chromosome distant from main metaphase. *c*, Same culture as *b*. Anaphase, note lagging of chromosomes.



Chromosomal behaviour during mitosis was studied in live cultures under phase contrast and in the original stained cultures from 1–45 days after exposure. The mitotic index was obtained by counting all mitotic figures and relating them to the total number of cells of each monolayer grown on the coverslips. Frequency of mitotic abnormalities was obtained by counting all abnormal mitoses and relating them to the total number of mitosis in each culture. DNA metabolism was examined by autoradiography and Feulgen microspectrophotometry^{2–4}.

Results were reproducible in all cultures. There were significant differences between control cultures and those exposed to smoke from marijuana and Kentucky Standard cigarettes (Table 1). From 1–4 days after exposure, cytotoxic effects such as pycnosis, necrosis and cell death were observed in the exposed cultures, accompanied by a striking decrease of mitosis and DNA synthesis. These early alterations were more marked after exposure to either whole smoke or the gas vapour phase of Kentucky Standard than they were after smoke from marijuana cigarettes. The lesser cytotoxic effect of marijuana, which contains a sticky resin, may perhaps be because cigarettes made from marijuana have a larger side-stream than Kentucky Standard cigarettes so that, in spite of the higher dose, less smoke *per se* reached the cultures¹. Other alterations observed in surviving cells were essentially the same after exposure to marijuana and Kentucky Standard cigarettes. There was a marked increase in size of cytoplasm, nucleoli and nuclei¹, accompanied by a significant increase in DNA content (Fig. 1). In control cultures even the largest nuclei contained 2–4 or only slightly higher DNA amounts, in all exposed cultures none of the large nuclei had 2–4 DNA, but the majority carried a significantly higher DNA content ($P=0.0005$). As can be seen from Table 1 and the example in Fig. 1, this striking increase in DNA was observed very early after exposure (1 day) at a period where mitosis and DNA synthesis were inhibited, and at later periods after exposure (7–45 days), at a period where stimulation of mitosis and DNA synthesis occurred (Table 1, Fig. 1). In addition, from 1–45 days after exposure, all cultures displayed mitotic abnormalities, which were not only more severe, but had also a significantly higher frequency than in control cultures. The abnormal behaviour of chromosomes during division was particularly clear in metaphase and anaphase, in which lagging and breakage of chromosomes and occurrence of tripolar metaphases and very large spindles were observed in living and fixed cultures (Fig. 2a–c). The difference in frequency of mitotic abnormalities in exposed and control cultures was particularly evident in young cultures. These control cultures had 0–3% abnormal mitosis, and the corresponding exposed cultures had 20–30%, that is a mean increase of about 7 times (Table 1). In older exposed cultures the significantly higher frequency of mitotic abnormalities persisted. Since, however,

the frequency of mitotic abnormalities increased with age in controls (up to 10%), the difference, although still being statistically significant, was somewhat less than for young

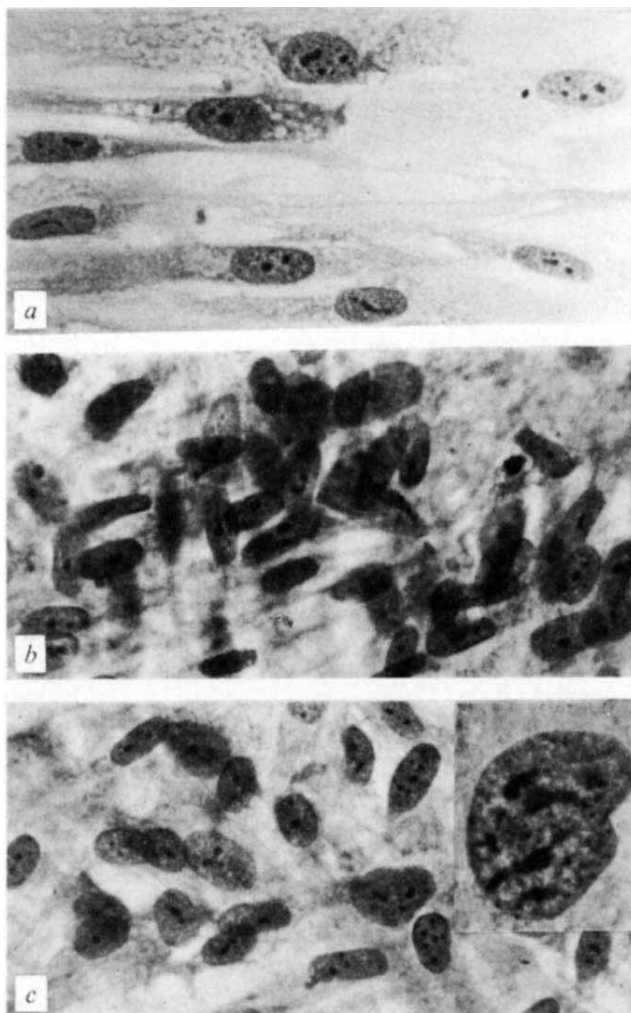


Fig. 3 *a*, Fibroblastic cells in monolayer in control culture of human adult lung explants (H and E, $\times 500$). Note well separated cells. *b*, Same culture as *a*, but 17 days after 4 exposures to 4 puffs of marijuana cigarette smoke (H and E, $\times 500$). Note irregularity and disparity of cells, and criss-cross formation. *c*, Same culture as *a*, 17 days after 4 exposures to 2 puffs of Kentucky Standard cigarette smoke (H and E, $\times 500$). Note essentially the same irregularity of culture as in *b*. Note also irregular shape of large nucleus, multiple nucleoli (inset $\times 1,000$).

Table 1 Comparison of Effects of Fresh Smoke from Marijuana and Kentucky Standard Cigarettes on Mitosis and Metabolism in Fibroblastic Cells of Adult Human Lung Explants ($n_1=200$)

Type of cigarette	Time after last exposure (in days)	Type and mean ratios of frequency of alterations							
		Mitotic index		DNA synthesis (³ H-TdR) (number of labelled cells)		exposed cultures		controls	
						of cells with high DNA content) $n_2=7,500$		Mitotic abnormalities	
		Decrease	Increase	Decrease	Increase	Decrease	Increase	Decrease	Increase
Marijuana	1–4	4 $\pm$ 0.2 pCo=0.001	—	3 $\pm$ 0.1 pCo=0.001	—	—	5 $\pm$ 0.5 pCo>0.0001	—	7 $\pm$ 1.7 pCo>0.0001
	7–45	—	2 $\pm$ 0.2 pCo=0.005	—	2 $\pm$ 0.2 pCo=0.005	—	5 $\pm$ 0.5 pCo>0.0001	—	3 $\pm$ 0.6 pCo=0.005
Kentucky Standard whole smoke gas-vapour phase	1–4	9 $\pm$ 0.2 pCo=0.0005	—	10 $\pm$ 0.2 pCo>0.0001	—	—	5 $\pm$ 0.5 pCo>0.0001	—	7 $\pm$ 1.5 pCo>0.0001
	7–45	—	2 $\pm$ 0.2 pCo=0.005	—	2 $\pm$ 0.2 pCo=0.005	—	5 $\pm$ 0.5 pCo>0.0001	—	3 $\pm$ 0.5 pCo=0.005

n_1 =No. of cultures examined. n_2 =No. of cells measured. *Feulgen microspectrophotometry.

cultures (Table 1). A detailed study of time sequential microscopic, ultrastructural and cytochemical effects of marijuana and tobacco smoke on young and old human adult and foetal lung cultures has been completed (Leuchtenberger, C, *et al.*, in preparation).

The marked decrease of DNA synthesis and mitosis observed early after exposure was followed at later periods (7–45 days) by an increase of mitosis and DNA synthesis (Table 1). In comparison with controls there was about a two-fold augmentation of these and this was accompanied by irregular growth, characterized by disparity in shape and size of nuclei and nucleoli, piling up, and criss-cross formations of cells. The appearance of exposed cultures was strikingly different from the corresponding control cultures, which showed rather regular monolayers with more or less contact inhibition (Figs. 3a–c).

It therefore seems that fresh smoke from cigarettes made from marijuana evokes essentially the same abnormalities in DNA metabolism, mitosis and growth in fibroblastic cells of human adult lung explants as does whole fresh smoke or the gas-vapour phase from Kentucky Standard cigarettes. This similarity is particularly noteworthy when the important role of cigarette smoke for pulmonary carcinogenesis is examined^{5,6}, but while it is tempting to suggest that smoke from marijuana cigarettes plays the same role in pulmonary carcinogenesis, no conclusions can be drawn until results of epidemiological studies in human marijuana smokers and chronic inhalation experiments in laboratory animals with marijuana cigarettes become available.

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Effect of Commensal Hydroids on Hermit Crab Competition in the Littoral Zone of Texas

In Galveston Bay, Texas, the hermit crab *Clibanarius vittatus* is the dominant animal on many shallow mud and sand flats. During the winter the hermits *Pagurus longicarpus* and *Pagurus pollicaris* migrate into the region dominated by *Clibanarius* and remain there until the following spring. Hermit crabs engage in ritualized fights¹ in competition for gastropod shells which are a necessary protection from predators. In both laboratory and field encounters, *Clibanarius* are very aggressive in competition for shells and are usually able to win shell fights

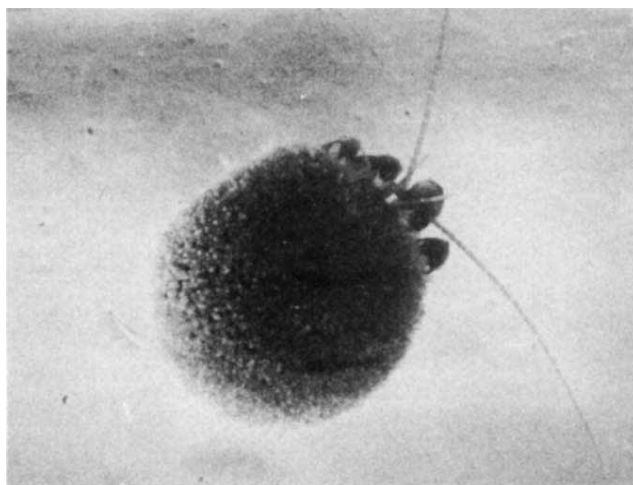


Fig. 1 *Pagurus longicarpus* bearing colonies of *Hydractinia echinata*. Note that any hermit occupying these shells will be in constant contact with the hydroid polyps.

with *Pagurus*, even if exceeded in size. As suitable unoccupied shells are extremely rare, *Pagurus* are at a potential disadvantage in competition with *Clibanarius* for this resource.

About 20–30% (depending on locality) of *Pagurus* collected bear shells with hydroid colonies, either *Podocoryne carnea* or *Hydractinia echinata* (Fig. 1). These hydrozoan colonies grow on the outside of the shells, secreting an intricate exoskeleton under which the stolons grow and from which the zooids arise. Of several thousand shells occupied by *Clibanarius vittatus*, one bore a healthy colony of commensal hydroids, several had regressing colonies, and others bore skeletal remnants of colonies. (The zooids of both genera of hydroids are readily destroyed by abrasion or other adversities, but living tissue remains in stolons under the skeletal mat. This tissue can regenerate the colony if fed and protected from exposure to air.)

Pagurus spp. usually actively avoid contact with *Clibanarius* in the field. In artificial encounters or when *Clibanarius* are first presented with shells covered with commensal hydroids, *Clibanarius* attempt the normal shell-examination² or shell-fighting ritual¹. When this species contacts the hydroid colony, all types of zooids (gastrozooids, ordinary dactylozooids, and spiral zooids) make the crab behave as if stung. Thereafter, the crab avoids the hydroid-occupied shell as long as it is left in the container. Thus inexperienced *Clibanarius* are repelled by neither visual nor olfactory cues from the hydroid. Repulsion is a result of individual experience. In an early experiment, one of the *Clibanarius*, deprived of its shell, did enter a hydroid-covered shell; but it soon used its heavily armoured chelae to pick off and discard all the zooids it could reach. The hydroids then could not reach the crab.

To demonstrate that *Clibanarius* prefer shells without hydroids, we removed ten *Clibanarius* from their own shells and offered each a shell covered with hydroids (Table 1). Eight of the ten crabs refused to enter the hydroid-covered shells during the 30 min trials. Each of the eight was then offered a clean shell. All eight entered the clean shells. The two crabs which had entered hydroid-covered shells switched to clean shells, one in 2 min 30 s, the other after 45 min. (The hydroids' stings thwarted attempts by the latter crab to leave or to investigate the clean shell.)

To be sure that the hydroid-covered shells were acceptable to crabs after as well as before the tests, twenty *Pagurus* (ten *P. longicarpus* and ten *P. pollicaris*) previously deprived of their shells were then offered the same hydroid-covered shells, and all twenty entered the shells. Only four of the twenty *Pagurus* switched from a hydroid-covered shell to a clean one when given that opportunity. Of these four, two shortly returned to their hydroid-covered shells.

Hydroids were observed to sting neither their *Pagurus* hosts nor other *Pagurus*. *Pagurus* occasionally eat hydroids covering a neighbour's shell. Although such feeding is obviously disadvantageous to the hydroid, it may allow *Pagurus* to become gradually immune in a manner similar to that postulated by Cantacuzene³ and subsequent authors for acquisition of immunity by *Pagurus prideauxii* to its actinian symbiont *Adamsia palliata*. Alternatively, the hydroid may contain a substance which inhibits its nematocysts from discharging and stinging other members of the colony. An occasional hydroid diet may provide *Pagurus* with such an inhibitor. As *Pagurus* do not aggregate in nature, feeding on hydroids borne by other *Pagurus* does not occur frequently enough to destroy the colonies.

Table 1 Response of Crabs to Shells with and without *Hydractinia echinata* Colonies

Species	Shells with hydroid Trial 1		Shells without hydroid Trial 2a		Shells without hydroid Trial 2b	
	Accepted by	Time (s)	Accepted by	Time (s)	Accepted by	Time (s)
<i>Clibanarius vittatus</i>	2 of 10	28	8 of 8	19	1 of 2 (see text)	30
<i>Pagurus longicarpus</i>	10 of 10	33			2 of 10	90
<i>Pagurus pollicaris</i>	10 of 10	19			2 of 10 (see text)	20

Each crab was tested individually in a small aquarium. Test was scored as rejection if crab did not enter shell in 30 min. "Time" is mean time from first contact to entrance. "Accepted by" is the number of crabs accepting shell out of the number offered shells. In trial 1, the crab was given shell with hydroid. In trial 2a, crabs which rejected hydroid-covered shell in trial 1 were given comparable shell without hydroid. In trial 2b, crabs which entered hydroid-covered shell in trial 1 were given comparable shell without hydroid.

Clibanarius with hydroid colonies on the backs of their shells (where the colonies could not sting the crabs) might be protected from losing their shells to other *Clibanarius*. The habits of *Clibanarius vittatus*, however, exclude the hydroids from such an association. At high tide, all three species of crabs studied may be found in water only a few centimetres deep. *Clibanarius* are frequently stranded at low tide, especially when north winds cause tides a metre or more below predicted levels. Both *Pagurus* species avoid exposure by moving to deeper water as the tide recedes. Only once was a straggler observed. The crab had dug a small hole not far from the water line so that its shell protruded only slightly above the water's surface. Even brief exposure to air can cause both hydroid species to drop their zooids. Both *Pagurus* and hydroids die easily on exposure to heat or foul water in the aquarium, while *Clibanarius* in the same containers survive and feed on the dead *Pagurus*. *Pagurus* avoid high temperatures reached in the shallow water of the bay flats (approaching 40° C) by a migration to deeper water during the summer months. Heat, exposure to air, and abrasion caused by the aggregation of large mounds of *Clibanarius* probably account for the dead hydroids found on some of their shells. It is doubtful that a colony could survive long on an inappropriate hermit host such as *Clibanarius vittatus*.

Hydroid colonies in Texas benefit their *Pagurus* hosts by protecting them from *Clibanarius*, and probably from predators—for comparison to *Pagurus bernhardus* see ref. 4. At least in the aquarium, the hydroid receives some food which is dropped by the crab, though the hydroid also catches zooplankton, which may be shared in turn with the crab. The work of Christensen⁵ on *Pagurus bernhardus* agrees with these observations, but his work is more extensive and is relevant to the European situation where he worked. Also in the aquarium, and probably in nature, hydroid colonies on shells not occupied by hermits are sometimes smothered by sediments. If a crab carried the shell, both its locomotor activity and respiratory

current would help keep the hydroid free of sediment. The association between the hydroids and *Pagurus* spp. is of advantage to both partners, a clear case of true mutualism.

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Epidermal Lesions in Irish Sea Flatfish

PERKINS *et al.*¹ suggest that the apparent increase of epidermal lesions in the flatfish of the north-east Irish Sea during 1971 may be associated with pollutants, particularly polychlorinated biphenyls (PCBs). They suggest that the simultaneous increase of lymphocystis, fin damage and ulcers represents an abnormal situation that may have resulted from the increased dumping operations carried out in the Irish Sea over the past few years.

In April 1972 we completed a similar survey extending over the area from St Bees Head to Liverpool Bay. We also found evidence of lymphocystis in dab, *Limanda limanda*; plaice, *Pleuronectes platessa*; and flounder, *Platichthys flesus*; the infection was restricted to the older fish and this we interpret as increased susceptibility at sexual maturity or, more likely, as a result of the increased number of encounters between fish which takes place during spawning aggregations, thus increasing the chances of transmission. Bacterial ulcers were also observed in these species but were not recorded in another twenty species. Partially and completely healed injuries were observed on the fin margins of the three flatfish species. This damage was almost certainly due to previous capture and subsequent escape from the trawl or rejection by fishermen. We saw no evidence of true fin rot.

The highest incidence of this type of damage occurred in the flounder, the least commercially important and therefore most frequently rejected of all the flatfishes. It is possible that infection had become established in a small number of these damaged fish.

Of 3,468 fish examined, the frequency of infection with lymphocystis in the dab and plaice (1.1 and 1.9% respectively) was similar to that recorded by Perkins *et al.*¹ (1.6 and 1.6%). For these species we found the incidence of ulcers (1.0 and 0.3%) and of fin damage (1.0 and 1.0%) to be significantly less than that recorded in 1971¹ (4.0 and 1.9% for ulcers; 1.9 and 2.3% for fin damage). Of the 397 flounders examined the incidence of lymphocystis, ulcers and fin damage was 14.6, 1.3 and 2.0% respectively, which we could not compare with Perkins's sample of 35 flounders.

If their data represented the coincident peaks of three epizootics in 1971 it seems from our survey that the frequency of fish with ulcers and fin damage declined in 1972. From records gathered by this Ministry during the operation of its scheme for the voluntary control of dumping, there was no corresponding reduction in the quantities of wastes dumped from vessels in this area.

It has been suggested by Brinkmann² that a high incidence of epidermal lesions is characteristic of many sea areas where salinity is low and the circulation somewhat restricted. Both lymphocystis and ulcers have been observed in Irish Sea flatfish for over fifty years^{3,4}, and it is commonly observed by both scientists and fishermen that the frequency of these infections undergoes cyclical fluctuations. Apart from any changes in the virulence of pathogens, these fluctuations are probably related to changes in salinity, water temperature and

year-class strength and we believe therefore that, of all the environmental variables which can be invoked to explain these results, pollution is the least likely.

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Courtship-feeding, Egg-size and Breeding Success in Common Terns

FEEDING of female birds by their mates is generally known as "courtship-feeding" because it is thought to play a role in establishing and maintaining a pair-bond¹⁻³. Its nutritional importance to the female at the time when she is forming eggs has been pointed out⁴⁻⁸, but no direct evidence for this function has been given.

In the common tern (*Sterna hirundo*)⁹⁻¹³, as in the closely related Arctic tern (*Sterna paradisaea*)¹¹⁻¹³, courtship takes place in three phases. In the first phase males carry fish around the colony and display to unmated females, but do not feed them regularly until the pair is fairly firmly established. In the second phase the pairs spend much of the daytime on the feeding-grounds, where the male frequently feeds the female; they return to the colony in the evenings. In the third phase the female stays in the pair's territory and is fed there by the male until the clutch is complete. In my study the third phase started between 6 h and 6 days before the first egg was laid. Once settled in the territory, the female spends most of her time there (Fig. 1), leaving it primarily to drink and bathe, catching little or no food for herself.

From May 21 to 28, 1971, I watched twelve pairs of common terns during the third phase of courtship in a colony at Bird Island, Massachusetts, USA (41° 40' N, 70° 43' W). Each food item fed to the females was observed from a hide at a range of 2-7 m. Fish (14% of items fed by frequency, 52% by weight) were identified in most cases to species, and their lengths estimated using the birds' bills as templates. Shrimps (48% by weight) could not be identified to species, but were assigned to one of five size-classes. The weight of each food item was estimated from length-weight curves compiled from samples of each species collected near the colony.

Feeding continued for about 14.5 h daily (0430-1900 EST): I watched for the first 9.5 h each day, and made at least two sample watches in each subsequent hour. There were no significant hour-to-hour variations in feeding rate when averaged over the 8-day period, so the 9.5 h samples included about 65% of the food fed to the females. Ranking the males according to the total amount of food brought, the rank order in the incomplete afternoon samples was very similar to that in the systematic morning samples; the latter are used in subsequent comparisons.

Designating the day when the first egg was laid as day *L*, most females laid their second egg on day *L* + 2 and their third egg on day *L* + 3 or day *L* + 4; two females laid only two eggs. Averaging the data for the twelve pairs, feeding-rates were highest between days *L* - 2 and *L* + 1, and dropped off sharply after day *L* + 2 as the males began to take a share in incubation

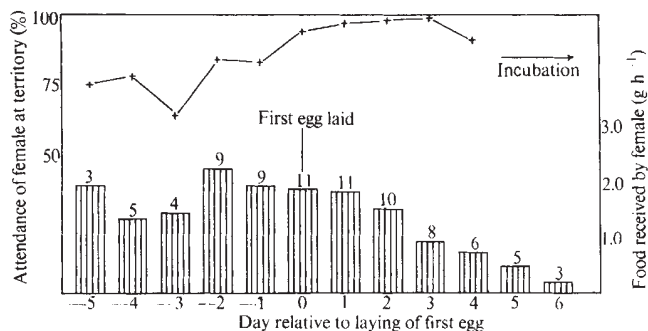


Fig. 1 Mean attendance of females at territory (+—+) and mean rate of feeding of females by males (histograms) during the egg-laying period. Sample sizes (figures above histograms) varied from day to day because of asynchronous laying. Data from females not yet settled (< 60% attendance) are not included.

(Fig. 1). An index of courtship-feeding performance for each pair was obtained by comparing the total biomass fed to the female with the average amount fed to all females on the corresponding days in the laying period. The most complete data were obtained for days *L* - 2 to *L* + 3 (Fig. 1), but the rank order of the males was not changed significantly by including data for the six pairs observed earlier; accordingly the indices listed in Table 1 are based on data for all days between *L* - 5 and *L* + 3. The resulting index of courtship-feeding performance was correlated with the total fresh weight of the clutch, and also with the fresh weight of the third egg (assigning zero to the "third egg" in two-egg clutches) (Table 2).

Altogether thirty-five clutches in the colony were marked and weighed at the time of laying. Chicks were marked at hatching and weighed daily until fledging or death. No eggs or chicks were taken by predators. In most broods chicks from the first two eggs fledged successfully, an exceptionally good performance for the species^{14,15}. Of thirty chicks hatched from third eggs ("C-chicks"), seven fledged: these hatched from eggs significantly larger than the remaining twenty-three third eggs (Table 2). Of the twelve pairs studied in detail, two fledged their C-chicks: these were ranked second and third in the male's courtship-feeding performance, marginally statistically significant ($P=0.089$). Thus fledging success, at least in C-chicks in a favourable year, is correlated with egg-size and probably also with the male's performance in courtship-feeding.

In a 1972 egg-exchange experiment, C-chicks hatched from large eggs survived much better than those from small eggs during their first four days of life, even when raised by parents that had laid small eggs. A similar result has been reported for herring gulls (*Larus argentatus*)¹⁶. In common terns, unlike herring gulls¹⁶, neither large eggs nor the large chicks that hatch from them appear to contain significantly more energy reserves in the form of lipids than small eggs or chicks (R. W. Risebrough and M. Gilbertson, unpublished data), but large chicks appear more vigorous than small chicks in competing for food with their older siblings; this might explain their better early survival in a year when food is short at the time of hatching. However, in 1971 only one C-chick died before the age of four days; most of the deaths occurred at ages of 8-13 days, when survival is expected to depend less on initial weight and vigour and more on subsequent parental care.

In nine pairs, including seven of the twelve studied during courtship, one of the parents was marked, and the total amount of food brought to the chicks by each parent was recorded (by the above methods) during at least one complete 24 h period between day *H* + 1 and day *H* + 4, where *H* denotes the day when the first egg hatched. At this period the male brings most of the food while the female broods the chicks¹², so the amount of food brought by the male is the most important index of parental feeding. The total weight of the brood on day *H* + 4 and the weight of the C-chick on its third day of life (usually *H* + 5 or *H* + 6) were selected as indices of success; by

Table 1 Indices of Breeding Performance for the Twelve Pairs Studied in Detail

Pair	Date of laying first egg	Index of courtship-feeding performance	Total weight of clutch (g)	Weight of third egg (g)	Food fed to chicks by male (g day ⁻¹)	Weight of brood, day H+4 (g)	Weight of C-chick, age 3 days (g)	Fledging of C-chick
A	May 22	1.47	61.8	20.3	33.1	85.0	13.5	No
B	May 24	1.25	72.5	23.4	62.2	102.0	24.0	Yes
C	May 25	1.24*	63.3	19.8	16.3	76.0	17.5	No
D	May 20	1.19	59.9	19.4	73.7	98.0	22.5	Yes
E	May 28	1.13	63.3	20.3	n.d.	96.5	22.5	No
F	May 25	1.10	43.2	none	n.d.	59.0	—	—
G	May 23	0.86	64.5	21.2	8.0	80.0	10.5	No
H	May 23	0.81	61.7	19.7	26.2	84.5	17.5	No
I	May 27	0.78	59.4	18.6	n.d.	91.0	21.0	No
J	May 26	0.70	56.8	19.6	n.d.	76.5	17.0	No
K	May 21	0.69	61.8	20.1	24.1	75.5	16.5	No
L	May 26	0.63	41.6	none	n.d.	33.5	—	—

* This female, unlike the others, was fed a significant amount of food by other males. Her mate was ranked sixth in the order of food brought by the males; this rank has been used in computing correlations with the feeding, weights, and fledging of the chicks.
n.d., Not studied at this period.

Table 2 Spearman Rank Correlation Coefficients between Indices of Breeding Performance

	Number of pairs	Total weight of clutch	Weight of third egg	Food fed to chicks by male	Weight of brood, day H+4	Weight of C-chick, age 3 days	Fledging of C-chick *
Courtship-feeding performance	12	+0.58 †	+0.51 †	+0.57 §	+0.66 †	+0.41 §	§
Total weight of clutch	35		+0.94 ‡	-0.43	+0.57 †	+0.59 †	
Weight of third egg	32			-0.20	+0.48 ‡	+0.60 ‡	†
Food fed to chicks by male	9				+0.84 ‡	+0.65 ‡	†
Weight of brood, day H+4	35					+0.65 ‡	†
Weight of C-chick, age 3 days	30						†

* Mann-Whitney U-test, comparing successful with unsuccessful pairs¹⁹.

† $P < 0.01$; ‡ $P < 0.05$; § $0.05 < P < 0.1$; || $P > 0.1$ (refs. 19, 20). All tests are one-tailed.

these dates the differences in weight between broods are much larger than the initial differences, and primarily reflect differences in growth. These three indices were all positively correlated with courtship-feeding performance and with the fledging success of the C-chick, but they were less consistently correlated with egg-size (Table 2).

Survival of C-chicks may thus be influenced by the performance of their fathers at two different times: at the time of laying, through the dependence of early survival on egg-size demonstrated in 1972; and in the period after hatching, through the dependence of later survival on parental feeding and early growth (Table 2). The males that performed well at both periods were those that switched most successfully from one group of food species to another: from shrimps and full-grown silversides (*Menidia menidia*) at the time of laying, to silversides fry and cunners (*Tautoglabrus adspersus*) at the time of hatching.

These results suggest a third function for courtship-feeding in common terns, in addition to the two above. The performance of the male in courtship-feeding is a predictor of his future performance in feeding the chicks (Table 2), in the period just after hatching when they are usually most critically dependent on him^{12,14,17}. It can thus be used by the female to assess his future performance as a parent. At least until the eggs are fertilized, the female has less time and energy invested in the nesting attempt than the male, and theoretically less to lose by breaking off the courtship if she finds indications of future incompetence¹⁸. The second phase in courtship could thus be used to test the male's competence in fishing, and the third phase to test his dependability in bringing food to the territory. Although I obtained no conclusive evidence that pairs broke up during these phases in courtship, I saw several females which settled in my study-plot for one or two days, but moved away after receiving relatively little food from their putative mates. During the first phase, incipient pairs break up very frequently,

as both males and females display to a number of potential mates⁹⁻¹³. The male's use of a fish in his advertising flights at this period may have more than symbolic significance: it indicates his future potential as a provider at the time when females are actively selecting their mates.

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BOOK REVIEWS

Science in an Eccentric Mirror

Science at the Crossroads. By Herbert Dingle. Pp. 256. (Martin, Brian and O'Keefe: London, September 1972.) £3.

THIS is Professor Dingle's account of his attempt to persuade the scientific community that the theory of relativity is wrong and should be repudiated. It is a singular, idiosyncratic, and in many ways outrageous book, but should not be dismissed as altogether worthless. The image that science presents to its experienced practitioners is well-rounded and almost without blemish; a reflexion of ourselves from a cracked and eccentric mirror may sometimes be healthy.

Professor Dingle's quixotic quest is for a valid answer to what he calls "the question". "According to the special relativity theory, as expounded by Einstein in his original paper, two similar, regularly running clocks, A and B, in uniform relative motion, must work at different rates. In mathematical terms, the intervals dt and dt' , which they record between the same two events, are related by the Lorentz transformation, according to which $dt \neq dt'$. Hence one clock must work steadily at a slower rate than the other. The theory, however, provides no indication of which clock that is, and the question inevitably arises: how is the slower-working clock distinguished?"

This is a perfectly reasonable question to which science should indeed give an answer. But first, what do we mean by "science" in this context? Professor Dingle strove mightily, by the magic of his pen, to charm such an answer from such high and mighty figures as the editor of *Nature* and several successive presidents of the Royal Society. But physics has no Pope with authority to proclaim doctrine. Several experts on relativity — especially Professor McRae — made reasoned replies demonstrating the fallacies in Dingle's objections; but he claimed that these were inadequate, or that they were formulated in mysterious mathematical language which did not correspond to any

genuine physical reality. The fact that other experts were reluctant to enter the lists against him was interpreted as a cowardly conspiracy of silence, symptomatic of a state of decadence in the intellectual integrity of science.

The charge of mathematical mystification is not altogether unjustified. Those theoretical physicists who discuss tachyons as if they were as familiar as boiled eggs, and assert the metaphysical principle "anything which is not prohibited is compulsory", deserve several bucketfuls of cold scepticism. The whole structure of modern physics is such a composite of mathematical theory and sophisticated observation that one is often at a loss to know whether any particular statement is logically necessary but empirically false or experimentally verified but theoretically anomalous. But never forget, oh best beloved, the positron, the neutrino, parity non-conservation, and the omega-minus, which were predicted in this spirit and subsequently observed.

He is also quite justified in expecting the professional experts to provide answers, if they can, to all questions concerning their specialties. Scientific knowledge is no more than what scientists know, and what they agree, one with another, to be the truth of the matter. Relativity theory is the responsibility of the relativists, in the first instance, and they cannot decline the challenge of debate, however sure they may be of their ground. In default of such champions, physicists generally must be willing to take up the cause, and at least reassure themselves, by public discussion, that their conventional opinions are soundly based. The scientific community has no machinery for delegating this responsibility to particular individuals. Professor Dingle's suspicions would be rightly inflamed if there had, as he claims, been an inadequate public reply to his objections.

But here, again, he is grossly unfair. The clock paradox, and its resolution, was discussed in detail by Einstein himself, and by many later scholars.

Glancing at my own bookshelf, I find Pauli's admirable treatise on Relativity Theory (originally published in 1921) which explains the position very clearly, and *Introducing Relativity* by W. G. V. Rosser, published in 1967, which devotes about 50 pages to this very topic, with particular reference to Dingle's arguments. In the library there is *Time and the Space Traveller*, by L. Marder (1971), which is entirely concerned with the theoretical analysis and experimental confirmation of relativistic time phenomena. Books such as these have been favourably reviewed, recommended as textbooks, and made the basis for undergraduate lectures in every university in the country. They have been worked over carefully without adverse comment by a large number of theoretical and experimental physicists who have been thoroughly alerted by one or another of Professor Dingle's numerous communications. In a community where honesty, integrity and mordant self-criticism are the norms of intellectual activity, this is as much of an answer as he can reasonably expect. The fact that he, one man in a thousand, thinks differently is scarcely a major flaw in the scientific consensus.

Yet there is always the necessity to doubt. Looking at the previous paragraph, I thought of another crank, Alfred Wegener, and of the geology textbooks that rejected continental drift for half a century. He, too, wrote some easily refuted nonsense in places on which the experts could happily pounce. The deeper and more secure our beliefs, the more difficulty and the greater the necessity for occasionally holding it possible that we are mistaken. I must say that I should rather like a simple but accurate answer to Professor Dingle's "question" — not at the level of showing up the elementary fallacies which he spins around it but as a serious issue of natural philosophy.

Not being active in research or teaching in this field, I can speak with little authority, but I would interpret the present position somewhat along the

following lines. (a) Experiment has amply confirmed that rapidly moving "clocks" (for example, radioactively decaying particles) seem to run slow as seen by a stationary observer. (b) Observers in different frames moving relative to one another would certainly disagree about whose clocks seemed to be running the faster. But special relativity cannot resolve the disagreement because it deals only with clocks (or assemblies of clocks or "observers" or coordinate frames) that are moving relative to one another with uniform velocity; clocks that have come close to one another at one instant of their respective careers can never come together again to check for disynchrony. (c) To make this comparison, the clocks must be accelerated (or decelerated) relative to one another according to some scheme that will bring them back into the same ball park. Each clock can then yield a number recording the interval of time between the two events of their initial and final meeting. (d) These two numbers need not be the same. The interval recorded by each clock depends upon its dynamical history between the two events. If their histories are not the same, then one clock will be found to have run more slowly than the other. This has been confirmed experimentally by taking an atomic clock around the world in a jet plane. (e) To distinguish the slower-working clock (as demanded by Professor Dingle) one must use the theory of *general* relativity, which takes account of accelerations. The dynamical history of a clock is represented geometrically by its world line in "space-time". A clock is itself, by definition, a physical system that integrates infinitesimal intervals, ds , along its world line. The axioms of the tensor calculus ensure that such an integral is well defined, and not arbitrarily dependent on the notion of an observer. But the integrated interval is not the same along every world line between two events. (Similarly, the distance from Bristol to London along the M4 is a well defined quantity, but it is not the same as the mileage that would be recorded in going up the M5 to Birmingham and then down the M1 to London.) (f) To avoid the use of this difficult mathematical language, one must introduce the "physical" concept of a gravitational field—a "fictitious" field of force observed by a physicist who does not realize that he is being accelerated. The simpler consequences of general relativity for the measurement of time can then be deduced phenomenologically from the principle that "a clock runs more slowly in a region of high gravitational potential". In the case of the elementary "twin paradox", for example, the chap that has felt the accelerations and decelerations of the

rocket flight to alpha-Centauri has aged less than his brother left on Earth, because his gravitational potential seems to himself much higher throughout the flight. (g) In fact, the answer to Dingle's "question" is simple: the fastest working clock between any two events is one that travels between them by free fall. (h) Hypothetical or actual experiments involving the synchronization of clocks are thus not in contradiction with the principle of relativity; they tell us nothing special about the velocity of light, the motion of the ether, absolute scales of time, or other ingredients of non-relativistic theories of space and time. Nor is there any evidence that our present ways of thinking about these things pose a greater danger for mankind (as Dingle asserts) than do any of our other cock-eyed, makeshift, ramshackle theories of the physical world. Interesting, subtle, and controversial questions arise when we try to think about the space-time metric of the whole cosmos, or set down the equations of physics near a "black hole"—but that was in another country: and besides (if we are to believe Dingle) the wench is dead.

For one reader, at least, this book has proved not altogether worthless. In its foolish, sincere, dishonest, wrong-headed, exasperatingly charming way, it has made me think further about the way in which "scientific knowledge" is validated, affirmed and made manifest in modern society, and about the foundations for my belief in the relativistic theory of space, time and gravitation. The fact that it has strengthened my confidence in the conventional wisdom at both levels need not be an objection to other people going through this same process. JOHN ZIMAN

Statistical Mechanics

Statistical Mechanics. By R. K. Pathria. Pp. xiii+527. (Pergamon: Oxford and New York, September 1972.) £8.

It is not, I hope, my most original remark to observe that the universe of knowledge is in a process of rapid expansion. The reason for this observation here is that the subject of statistical mechanics presents rather a good illustration. Just before the war, and into the 1950s, one had in Fowler's *Statistical Mechanics* a standard treatise. More up-to-date books at various levels of distinction and coverage were written as the subject attracted growing attention from physicists, chemists and also mathematicians, and Fowler's book is no longer a standard reference. Nonetheless, it is good to see that it appears in the bibliography of the book under review, together with the books by T. L. Hill, K. Huang, A.

Munster, R. Kubo and others. The time of a single standard reference in statistical mechanics has clearly passed for ever. Instead, the group of best books continues to grow. It is (fortunately) not exactly my task to place such books on some kind of ranking list. Suffice it to say, therefore, that the book under review belongs to this top group.

To any colleague who regularly reads the publications of the Hindustan Publishing Corporation of Delhi, this observation will come as no surprise. He will have noted (on their 1963 list) Pathria's first book on the theory of relativity, and will have observed that it attained a high level of clarity in the exposition of a difficult topic. Pathria's text on statistical mechanics has the same quality. It is careful, clear, offers many problems at the end of sections, and shows the author to be a wide reader who will refer from time to time to important or interesting topics which are sometimes left out of statistical mechanics texts. The theory of rotons, Chandrasekhar's limit and temperature-dependent energy levels are just a few examples. True, he has 507 pages on which to do so, but with this number of pages it is hardly the most voluminous book on statistical mechanics—a subject well known to resemble dialectical materialism in the size of volumes devoted to its study.

To indicate coverage: chapters 1 to 8 treat foundations and applications to ideal systems. Clusters, pseudo-potentials and second quantization as means of treating interacting systems follow in chapters 9 to 11. The concluding chapters deal with phase transitions, including a three page introduction to critical indices, and fluctuations.

The book contains rather little on the solid state or on melting apart from thermionic emission and the Debye theory, and not much on more chemical topics like mass action laws or reaction kinetics, though one problem is devoted to the Debye-Hückel theory of electrolytes. This is no criticism; authors must make selections. It would be unexpected and unlikely that this is the one occasion when the reviewer and the book are matched ideally, and there are accordingly some criticisms. The author's reference to Kappler 1931 for the study of the mean square deflexion of a galvanometer needle is rather old when the work of R. V. Jones and C. W. McCombie (in *Phil. Trans. Roy. Soc.* of 1952) is available, and I felt it to be a pity that his remarks about such basic questions as detailed balance and master equations were so brief, and almost incidental.

Returning now to the citation of Fowler's book, this appears with a publication date of 1955, whereas the last (second) edition was in fact published in 1936. It would, I think, be desirable if

at an appropriate international convention it were agreed that books which are reprinted should be cited in a bibliography not with the date of the reprint but with the date of the original publication. A citation giving a much later date of a reprint could be highly misleading.

Lastly, in his treatment of mass motion, after maximizing the entropy with Lagrangian multipliers α , β , γ for the moving system, the author assumes (top of page 153) that $\beta = 1/kT$, thus introducing the temperature of a moving object. From this essential step then follows the temperature transformation $T = T_0 \sqrt{1 - v^2/c^2}$. Most readers will not spot that this is the crucial step in Pathria's argument (which, in fact, I would not regard as unique and would prefer not to make at all), and it would have been worthy of a separate caution. In fact, it can be shown that if one assumes that thermodynamic relations are Lorentz-invariant then this temperature transformation follows. It would therefore have been sensible in such a discussion to raise the question of the Lorentz invariance of thermodynamic relations in the first place. Nonetheless, I believe that this is an excellent book from which to learn the methods and results of statistical mechanics.

P. T. LANDSBERG

Ecological Personalities

Philosophers of the Earth; Conversations with Ecologists. By Ann Chisholm. Pp. 201. (Sidgwick and Jackson.) £2.50.

ANY period of history, as observed by those living at the time, is coloured not only by the events that make up that period of history but also by the state of development that those events had reached when the observer first became aware of them. A sense of history and an awareness of the importance and relevance of the events that preceded those being observed is a necessary prerequisite if the full significance of the new events and the contribution being made by the players in these events is to be understood. Without these prerequisites the records made by an observer living through the period of history in question will suffer from distortion and be highly subjective. A journalist when reporting an event for the permanent column of a newspaper may up to a point be excused this background, but when he or she records in the permanent form of the book then, like the historian, he or she must try to see his or her heroes and the events they are influencing in a more critical light.

Miss Chisholm informs us in her foreword that she became aware of the word "ecology", and I assume the concomitant "environmental or ecological crisis", in the autumn of 1969 just be-

fore Sir Frank Fraser Darling's Reith Lectures on the BBC. This is somewhat surprising as the "crisis" had been news off and on for at least 10 years by that time and had reached an all-time peak during 1967 when the Torrey Canyon went ashore; however, it does explain her selection of "heroes", or what she calls Earth philosophers, and strange bedfellows some of them make. She is clearly influenced by the state the "environmental movement" had reached and by the personalities that occupied the centre of the stage at the moment that she became aware of it, but she has had the good sense to take advice and seek out some of those whose contribution, although no less important than that of the more popular figures, is perhaps less public. Each of us would, I am sure, have selected a different bunch of personalities but we would have had to include at least half of those interviewed in this book if we were to provide a critical review of the "environmental movement" of the past decade.

Unfortunately Miss Chisholm seems less aware of, or perhaps less interested in, the events themselves. She seems less informed about the events that preceded her awakening and incidentally that of many millions of other people too. For example, in the third paragraph of her foreword the author says "In America, as usual several steps ahead. . . ." Really? This is indicative of the sort of thing I referred to in my first paragraph. There is no doubt that the recent response of the USA to environmental wrongs has been significant but it has been a long time coming. Some of the countries in Europe have been doing things too and for longer. Where credit is due give it, but don't hand out laurels where they are not deserved.

However, Miss Chisholm's selection of heroes is interesting; some, as she admits, are not ecologists, in spite of the book's subtitle *Conversations with Ecologists*, but most of them have made major contributions to "environmental thinking". This use, or rather misuse, of the word "ecology" is one of the things troubling the real ecologists; it is rapidly becoming a debased word along with "environment", a situation which does nothing but add to the mounting difficulties facing the world community, and the journalists must take some of the responsibility for this debasement and the resulting confusion.

Miss Chisholm writes in a nice easy style but the question does arise in my mind, for whom does she write? If the layman is sufficiently interested in the "environmental movement" to want to know something of the characters involved, then all well and good, but I doubt whether the "environmentalists" or "ecologists", using the non-debased

forms, will find much in the *Philosophers of the Earth* to interest them. The treatment of necessity is too superficial and for the same reason the book will not be of much use to the historian—the absence of an index in any case makes the book next to useless for the researcher. Perhaps the author would have been better advised to select fewer and to delve deeper. In any event she has spared us yet another book on the "environmental crisis" but has focused on some of the personalities involved in the major events that historians will consider a revolutionary period of history.

ARTHUR BOURNE

Research Directory

Scientific Research in British Universities and Colleges, 1971-72, compiled by the Department of Education and Science. Vol. I: *Physical Sciences*. Pp. xxi+924. Vol. II: *Biological Sciences*. Pp. xxi+769. Vol. III: *Social Sciences (including Government Departments and Other Institutions)*. Pp. xxix+690. (Her Majesty's Stationery Office: London, 1972.) Vol. I £6.50. Vol. II £6. Vol. III £5.50.

THE 1971-72 edition of *Scientific Research in British Universities and Colleges* is still larger and more comprehensive than the previous issues. It is a unique and valuable work of reference, giving a nearly complete guide to current academic scientific and technical research. In addition, the Social Sciences volume covers research in Government and some other institutions, and this year includes Economic and Social History as a new subject heading.

The full subject and name indices enable the user to gain a fair indication of the scope of the research effort in any particular field, or to discover the current research interests of a particular department or individual. Every research worker should be familiar with it; those who are responsible for the organization or financing of scientific research cannot afford to be without it. It gives an overall view without which any research policy will be ill-informed and consequently misconceived.

I am aware of the enormous effort that must go into the publishing of this work, and would like to pay tribute to the compilers in the Office for Scientific and Technical Information; but now that it has grown so large, perhaps it is time for them to reconsider the scope and presentation of this work. What was suitable for the small, single-volume older editions is not really suitable to the present massive compilation. Faults or defects which were then not too serious are now more obvious and can present more difficulties to the user.

It is doubtful whether the separation

of subjects into three overlapping volumes is an advantage. It should be possible to present all the information in a more compact, yet still legible, form. Every relevant department, and, as far as possible, every individual should appear, with a clear indication of the main research interests of each person. The subject index is badly in need of improvement, perhaps by controlling the main index terms and greatly increasing the amount of cross-indexing. This presents great difficulties, but the compilers are in the Office for Scientific and Technical Information and might therefore be expected to do a little better. The present indices are somewhat lacking in both precision and accuracy.

Perhaps most important of all is a change in our attitude to works of this kind. All research workers (security considerations excepted) should consider it essential that details of their work should appear in the key directories. Those who finance their work might consider making this a condition of their grants. Only then will we have easy access to the facts we need to answer the many questions being raised today about the direction and utility of scientific research.

JEREMY WESTON

Experimental Prebiology

Origins of Life. By Cyril Ponnampereuma. Pp. 215+150 illustrations, 20 in full colour. (Thames and Hudson: London, October 1972.) £2.25 cloth; £1.25 paper.

THIS stands out among the welter of recent books on the origin of life by being the smallest and best illustrated to appear. It is a book designed for the non-specialist, and it is a pleasure to welcome it as a conspicuous success. Ponnampereuma, speaking with the full authority of one of the world's foremost practitioners in the field of experimental prebiology, has nevertheless been able to express himself in language that is readily comprehensible to the non-chemist. For many years he was with NASA in the exobiology division; he is now at the University of Maryland as director of the laboratory of chemical evolution.

Ten of the sixteen chapters are devoted to aspects of chemical evolution, and it is refreshing to read not only of the successes of the past, but also of plans for future experimentation. The other six chapters comprehend investigations of meteorites, and deal with other aspects of exobiology. Some of these chapters are highly speculative, and almost escape the boundaries of science. Perhaps there are better accounts to be found

elsewhere dealing with the fossil evidence, and with the possibility of intelligent life beyond the Earth. But there is no better account of the way that experimentation has illumined the processes of chemical evolution and our search for the origin of life.

The munificence of the illustrations can only be rivalled by other Thames and Hudson books in the same series. Chosen not by the author but by the publishers, they vary in relevance, and though they add much to the pleasure and interest of the volume, some are so inappropriate as to be misleading. What possible excuse can be found, for example, for illustrating a prebiotic site for the origin of life with a picture of the luxuriant vegetation of a tropical lagoon?

P. C. SYLVESTER-BRADLEY

Organophosphorus

Organophosphorus Chemistry. By B. J. Walker. Pp. 278. (Penguin: Harmondsworth, Middlesex, October 1972.) £3.

As a subject like organophosphorus chemistry grows, and itself begins to divide into specialisms—and to disappear into the decent obscurity of specialist journals—the single volume text becomes more than ever indispensable. Dr Walker's book is aimed at the interested graduate student and the final year graduate (may his tribe increase) who is taking organophosphorus chemistry as a special topic and has £3 to spend.

To cover so broad a field in so small a space an author must have very clear ideas on what to leave out, and here Dr Walker's judgment is sound. He provides a competent mechanistic treatment of the most important areas, at the expense of any systematic approach to synthesis in this field. There is a good account of the use of various spectroscopic methods in *Organophosphorus Chemistry*, and extended coverage of two topics which are clearly of special importance. These are the Wittig reaction, where the author is expert and produces an excellent review, and phosphorus in biological processes, where he is not. This last is the weakest chapter in the book, and ranges over biological energy transfer and the structures of DNA and the various RNAs (including a reference to something called the codon), without any real attempt to relate them to the organophosphorus chemistry involved.

The author also falls foul of SI units. He gives bond lengths in metres, but the molecules unfortunately come out only one tenth of the usual size. After page 141 Angstrom units appear, with a correct length in metres given also; with the curious result that the P-C bond

in ylids appears to be ten times longer than all the other bonds in the molecule. These are not typical, however, of a book which is fairly free of typographical errors and is attractively printed and produced. Sets of examples appear at the ends of chapters, and the bibliography is a model for any author, organized by topic, with a helpful brief description of each reference cited. The book can be recommended to the students for whom it is intended, and to libraries catering for them.

A. J. KIRBY

Chemical Lists

Chemical Substructure Index 1971. Annual Cumulative Edition. Part 1: Substructure Index A-Q, Users' Guide. Part 2: Substructure Index Q-9Y, Peptides. (Institute for Scientific Information: Pennsylvania and Uxbridge, Middlesex 1972.) N.p.

In his searches for information from secondary journals, the average chemist depends largely on *Chemical Abstracts* with its wide area of coverage and its various indexes. But these virtues carry a penalty difficult to avoid—the time interval between publication in a primary journal and the appearance of the corresponding abstract.

To reduce this interval for some journals, the Institute for Scientific Information publishes *Current Abstracts of Chemistry* and *Index Chemicus*. It also provides other aids based on the same material, including computer tapes, programs, and printed lists, enabling information services of varying degrees of complexity to be set up by users themselves, according to their needs. One of these aids consists of a listing of all chemical compounds enumerated in *Current Abstracts of Chemistry*, the *Chemical Substructure Index (CSI)*, which is produced monthly and cumulated annually to give the volumes under review.

The compounds are encoded into the Wiswesser Line Notation (WLN) and then listed alphanumerically in a permuted form under each significant symbol. There is also a "Quikscan" feature which indicates the presence of such symbols, although not their order. From this information, individual compounds or many part structures may be identified. In an attempt to ensure that search methods used will be correct, the listing is preceded by the notations for "Frequently Found Substructures", and by a *CSI Users' Guide*.

The encoders have produced notations of a good standard, and settle any problems arising in this connexion with WLN experts in the Chemical Notation Association, so that adverse criticism of the listing itself is not justified.

The preceding dictionary of substructures and the guide for users are in a very different category, however. Many of the notations in the dictionary are misleading or inaccurate. There is no indication as to which way a group is written to start a notation. One way of denoting an isothiocyano group and the notation for any azoxy group are incorrect, although only the correct form appears in the subsequent index. Some alternative ways of denoting branched structures are omitted.

The *Users' Guide* also depicts a situation which does not exist in the main index. Some examples of listings are shown lacking alphabetization after the initial grouping, and a wrong notation appears in one section.

Most of these errors do not affect the main body of the work, and probably indicate no more than that a thorough overhaul of the *Guide* is long overdue. But they do show how wrong it would be to attempt to use non-technical effort for search procedures, even though this is recommended by the *Guide*. The problems could be largely avoided by the use of WLN expertise, if only of a minor order, and this would avoid answers which result in an unjustifiable lack of confidence in the whole method. Considering how admirable the objectives behind the production of these volumes, and how well they are used by some organizations, such a conclusion would be as regrettable as it would be false.

P. A. BAKER

Insect Enthusiasts

American Entomologists. By Arnold Mallis. Pp. xiv + 549. (Rutgers University: New Brunswick, New Jersey, October 1971.) \$15.

THIS book contains biographical accounts and photographs of 206 entomologists of the United States and Canada. The subjects are roughly classified according to their spheres of interest. Among the pioneer entomologists the name of Thomas Say (1787–1834) is well known from the number of species he named and described. He was a great traveller and collector. T. W. Harris (1795–1856) combined systematic entomology with advice to farmers on the new pests they were continually discovering—all as the spare-time job of a conscientious and over-driven librarian at Harvard. It is remarkable how many later entomologists were inspired by his *Insects Injurious to Vegetation*. The first of the professional state entomologists was Asa Fitch (1809–1879) appointed by the New York State Legislature in 1854 to produce a long series of reports on noxious, beneficial and other insects in the state of New York. "I sometimes think," he wrote, "that there is no kind of

mischief going on in the world of nature around us but that there is some insect at the bottom of it." Thomas Glover (1813–1883) was the first federal entomologist, appointed in the Bureau of Agriculture in 1854. He was succeeded in 1878 by that colourful figure C. V. Riley (1843–1893): a gifted entomologist, wholly self-taught, intensely self-reliant, producing detailed descriptions of life histories, devising new methods of control and building an organization that was to become a most successful undertaking. His life has been well documented from many sources: his ambition and self-aggrandizement, his habit, like many others of the time, of appropriating the discoveries of his juniors, were well known—but failed to obscure his tremendous accomplishments.

L. O. Howard (1857–1950), whom many of us remember well, was brought up in this difficult school and has given a racy and good humoured account of it. He succeeded Riley at the age of thirty-seven in 1894; and under him the Bureau of Entomology came to maturity and the stature of the applied entomologist in the United States rose to a level that is difficult for us in Great Britain to appreciate. Howard was trained under J. H. Comstock (1849–1931) who had been more or less orphaned at the age of four, had very little schooling and a severe impediment in his speech; but after discovering Harris's *Insects Injurious to Vegetation* he developed a passion for entomology. Under pressure from his student contemporaries the college authorities permitted him to give a course in entomology in his sophomore year at Cornell—where he remained for sixty years and built up the leading teaching centre for entomologists in the United States.

Comstock is classified as a notable teacher; and so is E. O. Essig (1884–1964). Essig worked his way through college and became a brilliant and popular teacher. He was devoted to California—as one who accompanied him through the forests of the High Sierra could not fail to appreciate. He wrote a *History of Entomology* in 1931 with over one thousand pages—which referred to nothing outside California! But one learns in this book that the publishers were responsible for omitting the words "*in California*". It may be that Essig did not see much difference. G. F. Ferris (1893–1958) and R. E. Snodgrass (1875–1962) also appear as notable teachers. The students of Snodgrass are, of course, the entomologists of the world. He had a chequered career at the outset, losing appointments in entomology it would seem by reason of a propensity for practical joking; and was forced to take up commercial illustrating and odd jobs, until he was hired by L. O. Howard and given a post,

primarily as an illustrator, in the Bureau of Entomology. There he remained for most of his life, devoting most of his time to insect morphology—a strange official anomaly for which we may be eternally grateful to L. O. Howard. He was not always tied to the microscope; I remember him as a splendid guide in the woods of Maryland.

So the story continues. A most fascinating book for entomologists to dip into. Some subjects, of course, are better documented than others. G. C. Crampton (1881–1951) is underestimated. A curious omission is the name of W. T. M. Forbes of Cornell, who was publishing extensively at the beginning of the century; but being still alive he did not qualify for entry when this book went to press.

V. B. WIGGLESWORTH

Dental Scrapbook

Dental Morphology and Evolution. Edited by A. A. Dahlberg. Pp. x + 350. (Chicago University: Chicago and London, 1972.) £9.

PROFESSOR DAHLBERG has ably edited this volume and has provided his own fulsome review as the introduction. I must confess to a certain unease about this book in view of some of the extravagant claims made for it. Perhaps the strongest impression was the sense of *déjà vu*. Much of the work included has been overtaken by events. There have been a number of major symposia and books on comparable topics in the last few years most of which are more up to date than Dahlberg's.

The reason is not far to seek. The papers published in *Dental Morphology and Evolution* were delivered at a symposium in 1968; they reached me in the summer of 1972. Such an unconscionable delay in publishing has rendered what might have been a valuable contribution in 1969 significantly less so in 1972. Advances in the study of teeth move too fast for such a delay to be tolerable. The publishers have rendered a disservice to the authors, some of whom are understandably bitter over this delay. Indeed one author has vouchsafed the intelligence that his paper should not have been published now!

The articles by Turnbull and Clemens have been overtaken by the *Linnean Society Symposium (1970) on Early Mammals* (published 1971). Likewise the paper by Hiiemae and Crompton is the precursor of the major contribution by the same authors submitted for publication in 1969 and published in 1970.

The electron-scanning microscope study by Boyde entitled "Comparative Histology of Mammalian Teeth" is interesting as far as it goes but it does not go very far. Alan Boyde's flood of

further work renders this contribution something of an aperitif after the meal. Likewise, Poole's "Introduction to the Phylogeny of Calcified Tissues" has been dealt with by other authors (including Poole himself) in greater depth elsewhere. The impression is given that it was a précis of the major papers of Orvig and Poole published in 1967.

Papers 12, 13, 14, 15 and 16 seem to have little significant contribution to make. Hershkovitz's attempt at a new dental morphological terminology is valiant but by its very nature doomed. Something must be done sometime, but yet another plethora of cumbersome terms simply perpetuates the appalling reputation that dental "cuspologists" or "cristalogs" have acquired for themselves.

Tonge's review on the role of the mesenchyme in tooth development is welcome and Elwyn Simon's on Oligocene and Miocene Old World Anthropoidae is valuable.

Yes, there are a few good things in this volume and they could have been wholeheartedly welcomed a few years ago.

But even if this volume had appeared as long ago as 1969, it would most probably have received some not over-enthusiastic reviews. The blurb and the introduction read splendidly and are most impressive. The actual contents, in contrast, are a disappointment. This volume is a scrapbook of bits and pieces of varying quality. There is no real link even within the separate sections. Take "Phylogeny" for instance; Turnbull and Clemens have related contributions, the rest bear no relation to each other and precious little to phylogeny. In the final part "Morphology", Dahlberg has contributed an article which discusses the "elusive aspects of dental traits in respect to their penetrance and expressivity"—an apt summary of this volume.

L. B. HALSTEAD

Study of a Scavenger

The Spotted Hyena: a Study of Predation and Social Behaviour. By Hans Kruuk. Pp. xvi+335. (University of Chicago: Chicago and London, 1972.) £6.75.

BECAUSE they compete with man, many carnivores are unpopular, the ungainly spotted hyena occupying pride of place as the most despised of all—at least in popular imagination. Even in some recently published general works the picture of a ghastly cowardly scavenger persists. There might seem to be very little to be said in favour of the spotted hyena.

Hans Kruuk, who studied this animal as part of a wider programme of research initiated by the Serengeti Research Institute in Tanzania, has shown

how misleading this proverbial image really is. His book is an account of a carefully planned and thoroughly executed research project which will appeal not only to carnivore enthusiasts but also to those concerned with the broader aspects of mammalian ecology; to the palaeontologist; even to the sociologist.

Kruuk left few avenues of investigation unexplored. He observed hyenas by aeroplane and he followed them on the ground both by day and by night. He fired immobilizing drugs and marked representative animals for subsequent re-identification; and he examined their teeth while they were drugged. He fitted a radio transmitter to one hyena and kept it under continuous observation for twelve days; and he studied where the hyenas lived, in one lair becoming covered with fleas in consequence. He studied the relationships of hyenas to one another, to other carnivores and to their prey. He estimated the prevalence of sick prey animals and examined hyena droppings to find out what had been eaten. His wife also took part in the studies, fostering a baby hyena which used the Kruuk house as its den.

The story which emerges—packs of hyenas chasing and killing zebra and wildebeest; lions scavenging from hyenas; and lionesses chased up trees by hyenas—contrasts sharply with established ideas. Perhaps the most important observation is the great adaptability which the spotted hyena is shown to have in relation to its environment. Kruuk found that in the Serengeti National Park hyenas travel great distances in order to keep up with migrating wildebeest with consequent high mortality among cubs left unattended by their mothers. The hyenas in the nearby Ngorongoro caldera, however, were found to be an enclosed sedentary population divided into eight "clans" with clearly defined territories and with lower cub mortality. In contrast to the hunting hyenas of these two areas the hyenas living around the walled town of Harar in Ethiopia have become adapted to a scavenging, almost symbiotic, coexistence with man.

This great adaptability which Kruuk has demonstrated is of far reaching significance. In addition to its more obvious application to animal management in Africa today it opens a new field of study for the palaeontologist with evidence already before him that the spotted hyena once spread to Yorkshire and Ireland, with such varied diet as the interglacial hippopotamus and the woolly mammoth and migratory reindeer of cooler times.

The book is well laid out with clear headings, a good index and a valuable bibliography. There is only one cause for regret. The publishers have bound all the half-tone illustrations together

as one signature and have not done full justice to the author's brilliant photographs.

This is a remarkable and important book which will attract a diverse readership.

A. J. SUTCLIFFE

Purpose in Behaviour

Purposive Explanation in Psychology. By M. Boden. Pp. ix+408. (Harvard University: Cambridge, Massachusetts; Oxford University: London, August 1972.) £6.50.

THERE has always been a rift in psychology between those who insist that human behaviour can be understood only in purposive terms and those who refuse to acknowledge any but causal-mechanical explanations as scientifically admissible. This book is an attempt to heal that rift by showing us that we can continue in good conscience to be teleologists about human beings, indeed we can scarcely avoid doing so, yet, at the same time, we can recognize that we are physical mechanisms and that ultimately all psychological phenomena are caused by brain processes. The author introduces a valuable distinction here between "strict reducibility" which would imply that all the concepts which appear in the one language must be translatable into those belonging to the other language, so that the former is, in the last resort, redundant, and "empirical reducibility" which requires only the possibility of there being bridging laws which put the two in correspondence. It is this latter benign kind of reductionism which, according to the author, obtains between psychology and physiology. Much of her book is devoted to a detailed examination of the psychology of William McDougall who, though not much read these days, remains historically the arch-spokesman of the teleological approach in psychology. She defends her thesis by showing that, contrary to what McDougall himself believed, his approach was not incompatible with an empirical reductionism. She supports this by appealing at length to the new science of artificial intelligence where the same dichotomy reappears as between the purposive aspects of the programming and the deterministic aspects of its physical embodiment in an electronic machine.

The book is a powerfully argued and welcome contribution to the growing body of literature of recent years dealing with the philosophical foundations of psychology. However, the author's treatment of the mind-body problem which was so central for McDougall is, I think, marred by certain philosophical preconceptions which prevent her from doing justice to the dualist-interactionist position. JOHN BELOFF

INSTRUMENT REVIEW

Laser Velocity Measurements

Laser Interferometer 3SLVI-204 (Systems, Science and Software), from £3,300.

EVER since Newton discovered the interference rings which bear his name, optical interferometers have been used to measure optical distances by fringe counting. Until the 1960s these interferometers were used with conventional light sources, and all suffered from the short coherence length of the light, which meant that only small path differences (up to 30 cm) between the two component beams of the interferometer could be measured. This distance is estimated from the line width of the source (0.01 Å).

The introduction of lasers, with line widths a factor $\sim 10^{10}$ smaller, increased the coherence length to many kilometres and enlarged the path difference that can be used in an interferometer to the same distance. For example, an interferometer (Fig. 1a) may be used to measure a change in position of a distant reflector by counting fringes in the combined beam. The instrument is commonly known as a displacement interferometer and produces a fringe change of $2s/\lambda$ where s is the distance moved by the reflector. Velocity can also be measured with this instrument by measuring the rate at which fringes move across the detector. Currently available amplifying and recording equipment, however, limits the fringe rate that can be handled to ~ 100 MHz and in consequence limits the velocity that can be measured to ~ 0.03 mm μs^{-1} .

To overcome this limitation Barker (*Fine Structure and Release Wave Shapes in Aluminium by the Velocity Interferometer Technique*, SC-DC-66-2447 (Sandia Laboratory, Albuquerque, New Mexico, 1966)) has suggested that using the Doppler effect an interferometer could be made to measure velocity directly by splitting the returned beam

from the reflector and combining it again with one component delayed (Fig. 1b). Then the fringe change between two states of constant velocity is $2lv/\lambda c$, where l is the delay leg length, c the velocity of light and v the change in reflector velocity. The quantity $(\lambda c/2l)$ is the fringe constant, conveniently measured in units of mm μs^{-1} fringe $^{-1}$. This instrument is commonly known as a velocity interferometer, but it is as well to note that the quantity measured by the instrument is not exactly velocity; Clifton (*J. Appl. Phys.*, **41**, 5335; 1970) has discussed the approximations involved. There is no limit to the maximum velocity that can be measured, for, in practice, the delay leg can be made as short as is required. The lower limit, set by the fact that a long delay leg is required for small fringe constants, may necessitate separate mounting of the reflector prism, and shake in the return beam caused by floor vibration can be excessive. Any user contemplating a velocity interferometer with a fringe constant less than 0.01 mm μs^{-1} fringe $^{-1}$ should consider isolating both interferometer and reflector prism from floor vibration. Alternatively, if slightly larger fringe constants can be used, it is our experience that the optical path of the delay leg can be folded within the base of the interferometer using an extra prism. No special isolation from vibration is necessary for this folded delay leg or indeed for a normal interferometer mounted on a single platform. Target shake induced by floor vibration is not important in a velocity interferometer, but may be troublesome in a displacement interferometer.

The Systems, Science and Software SLVI-204 (S³-204 for short) can be operated in either displacement or velocity mode and is designed specifically for the measurement of surface velocities from shock experiments. This review is not the place to discuss shock phenomena, which are well described in the literature (for example, McQueen, R. G., Marsh, S. P., Taylor, J. W., Fritz, J. N., and Carter W. J., in *High Velocity Impact Phenomena*, edited by Kinslow, R., p. 294, Academic Press, 1970). Suffice it to say that surface velocity is closely related to the internal particle velocity and gives valuable information on the behaviour of the material. The S³-204 in the velocity mode measures surface velocity up to 3 mm μs^{-1} for a minimum time of 100 μs and with a time resolution

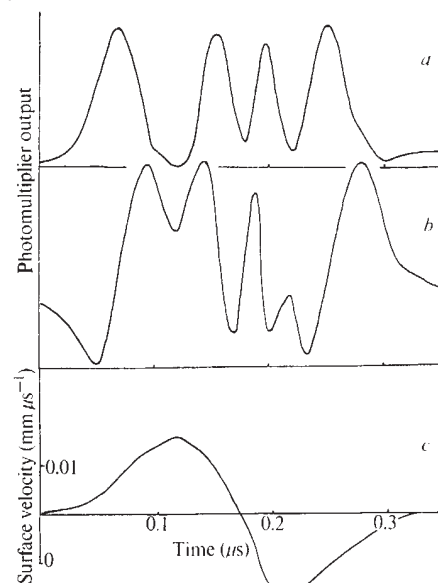


Fig. 2 Interferometer records. *a*, Photomultiplier signal (normal interference); *b*, photomultiplier signal with phase shift of $\lambda/4$; *c*, deduced surface velocity.

down to 2.5 ns, covering most of the requirements for shock experiments in dense materials induced by flyer plates, explosives, pulsed electric currents and pulsed electron beams.

The Spectra-Physics 1 mW HeNe laser supplied with the interferometer oscillates in the TEM₀₀ mode, is polarized, and produces only one axial mode. The laser is stable enough 30 min after switching it on for someone with a little practice to produce a single fringe over the whole laser spot. The laser power is adequate for polished targets, but a higher power would be necessary for poor, scratched or diffuse reflectors. The piezoelectric pulser supplied with the instrument is a considerable help in setting up and the dual pulsed photomultiplier assembly, although linear up to a few V only, operates reliably and consistently.

These are some areas of difficulty not specific to the S³ interferometer but inherent in the use of laser interferometers in general. First, the shocked target may tilt, moving the reflected beam away from the photomultiplier. The S³-204 can accommodate a lateral shift of 5.0 mm in the return laser beam, which with a 500 mm objective lens, corresponds to about 0.5 degree in target tilt. Objectives of shorter focal length can be used, but care should be taken in their selection, for the choice of lens is a compromise between tilt tolerance, recording time and surface

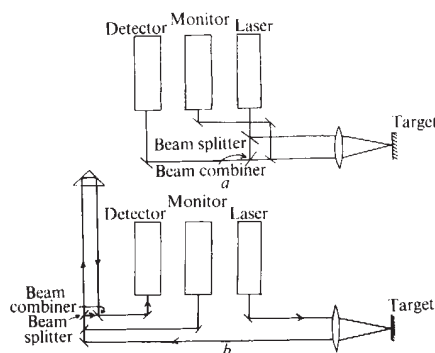


Fig. 1 *a*, Displacement interferometer; *b*, velocity interferometer.

velocity. S^3 offers a design service for objective lenses.

Second, electrical interference on the recorded signal increases the error in the velocity inferred. It is difficult to be precise as electrical interference is highly variable from one location to another, but for most locations the S^3 -204 is probably adequately screened. If, however, as was our experience, the interferometer had to be operated outside a conventional screened room near equipment generating severe electrical interference ($>1 \text{ kV m}^{-1}$) then the screening of the photomultiplier assembly is not adequate and local elec-

trical double screening is necessary to reduce the interference to an acceptable level of less than 10% of the recorded signal.

Third, the interferometer does not measure the direction of the target movement, and it is in many cases not obvious from the record itself where acceleration reversals have taken place. Nonetheless, the S^3 -204 can be adapted to use an optical technique of phase shifting the laser light recorded in one photomultiplier by $\lambda/4$ to obtain records in both photomultipliers that unambiguously identify acceleration reversals (Bouricous and Clifford, *Rev. Sci. Instr.*,

41, 1800; 1970). Fig. 2 shows interferometer records obtained with this technique together with the inferred velocity. In only one record are the acceleration reversals unambiguous. The technique is available from S^3 .

The S^3 -204 interferometer measures reliably surface velocities from shock physics experiments, and, once a prospective purchaser has decided which of the many available options are useful to him, the instrument will provide a useful addition to any laboratory working in shock physics.

P. H. WHITE
R. H. GOBBETT

CORRESPONDENCE

Creation or Evolution

SIR,—The discussion by advocates of creation vs evolution (*Nature*, 240, 365; 1972) was triggered by the "school textbook controversy" in California. Solan, at least by implication, identifies the dispute with the State of California, but a bill requiring Michigan's public schools to teach the biblical account of creation along with evolutionary theory was unanimously approved by the House Education Committee in Michigan on December 5.

The textbook controversy has both religious and scientific components. The religious arguments used by the California State Board of Education and its creationist supporters were opposed by eminent clergymen, representing several of the leading faiths, in the hearings at Sacramento on November 9. Other objections raised by the "creationists" were directed against the scientific content of evolution. These objections are uninformed, illogical, trivial, and, if they find their way into the textbooks, they will degrade the teaching of science to the children of California.

Dr John Ford, the vice-president of the Board, favours "as the best current explanation for variation among plants and other living things the Special Theory of Evolution as defined by G. A. Kerkut and others". He also states that science "classically ignores" the areas of "value systems, morals, art and poetry". Evidently, he does not know, or ignores, the contributions made to human welfare by great scientists who were also great humanitarians.

An engineer named Vernon Grose has been the Board's leading adviser on evolution. The Board elevated him to the State Textbook Commission. He is also a member of the Governor's Commission on Law Enforcement. Mr Grose's statements have made it evident that he does not have even a

rudimentary knowledge of biology. He says that Pasteur's demonstration that bacteria do not arise spontaneously disproves theories of the origin of life. Mr Grose strongly opposes the concept of adaptation as an evolutionary force. He asserts that "the regular absence of transitional forms may best be explained by a creation theory", and he dislikes the concept that plants and animals have a common ancestral origin. However, he has not so far proposed a revision of the primary sequence of cytochrome c.

The controversy continues, and the Board, on December 14, evidently favoured introducing the teaching of creation into science textbooks. There will be more news in January, following action by a new committee of the Board that includes Dr Ford and two scientists (Richard Bube and Robert Fischer) who are active in the religiously-oriented American Scientific Affiliation.

Yours faithfully,

THOMAS H. JUKES

University of California,
Berkeley, California 94720

SIR,—It is welcome that you are initiating a review of any time-honoured scientific tenet of faith—for this is what evolution has become to many of us—rather like a theological doctrine, to be defended with some passion. It is a good thing for people to question broad philosophical assumptions and keep open minds. On the issue of evolution as a primary directive force in the cosmos, I propose to do just that whilst feeling free to use the evolutionary hypothesis in relevant cases in my research and thinking.

I feel undogmatic and somewhat sceptical in the debate. Special creation cannot be proved, and a thorough-going evolutionary origin on the basis of environmental adaptation explains

much, but leaves plenty of unanswerable queries.

My atheist undergraduate diet of Haldane, Huxley, Bernal, Wells and so on failed to satisfy me that evolution is the sole creative force behind the cosmos and the biosphere and modern *Homo sapiens*. On more recent review, I find it too much for my credulity, for reasons similar to those cited by Vanderkooi and Van Kley (*Nature*, 240, 365; 1972). On the other hand, I find God real and His activity demonstrable over the years.

One demonstrates biogenetic evolution in the laboratory and field at species and generic level, but I doubt the validity of extrapolating these data and turning them into a first cause. Wouldn't we beat our students about the head for far less a sin?

So one opts for some creative power—I call Him God—as a first cause, which I maintain is common sense and fair science. Why did it happen—that is a non-scientific issue. How it happened is a valid question, but too difficult, except as the answer comes clear here and there from observation and experiments.

Yours faithfully,

DAVID ALLBROOK

Department of Anatomy,
The University of Western Australia

SIR,—I thank you for publishing my letter on the subject of creation or evolution.

The whole fable of evolution is nothing more than a confidence trick on the part of the Devil, who is as perfectly well able to blind the eyes of men of science as he is those of lesser mortals. A theory which results in a grovellingly debased view of human origins—the absolute opposite of the truth that man was created in God's own image—and which helps to spawn such

grotesquely distorted pieces of literature as Marx's *Das Kapital* and Hitler's *Mein Kampf*, can only have originated from such a source.

Yours faithfully,

ALAN RADCLIFFE-SMITH

13 Argyle Avenue,
Hounslow,
Middlesex

β -Carotene

SIR,—The following comments may be of some interest with respect to the letter of Drs Johnson and Fusaro.

While it is true that Kesten (not Keston) was the first to describe the use of β -carotene cream as a photoprotective agent, Mathews-Roth and associates were the first to show unambiguously in a number of patients and animals the effectiveness of internally administered β -carotene¹. It should be recognized that there is a marked difference between topically and internally administered β -carotene. As Kesten showed in a single patient, topically applied β -carotene provides some protection against photosensitivity. This protection is due to the absorption of part of the incident visible light by the carotene, and hence topically applied black treacle would probably have a similar effect. When β -carotene is taken internally, its protective effect is enhanced and it seems likely that the carotene is acting not merely as a light shield but as an *in vivo* quencher of singlet oxygen. Although Kesten did administer β -carotene orally to one photosensitive patient, she did not report any definite conclusions regarding the effectiveness of the treatment.

Yours faithfully,

ANTONY F. McDONAGH

School of Medicine,
Department of Medicine,
University of California,
San Francisco

¹ Mathews-Roth, M. M., Pathak, M. A., Fitzpatrick, T. B., Harber, L. C., and Kass, E. H., *Trans. Assoc. Amer. Phys.*, **83**, 176 (1970).

Congress Caveat

SIR,—We would like to reply to the letter (*Nature*, **240**, 428; 1972) about the prices of some of the excursions arranged for participants in the ninth International Congress of Biochemistry. When preparing for the Congress, the organizing committee asked a well-known Swedish travel agency, RESO, to arrange a number of visits and excursions in connexion with the Congress. The tours were to be arranged at RESO's financial risk and on the condition that they could not be cancelled on the basis of a low number of participants. The negotiations with RESO resulted in plans

for those visits and excursions that are described in circular 2 of the Congress. The arrangement of these tours constitutes no source of income for the Congress but should be considered only as a service to the Congress members. The tours specially arranged for participants of the Congress provide good opportunities for informal discussions and exchange of ideas, in combination with a comfortable way of doing some sightseeing. There is no doubt that the sightseeing by itself can, in many cases, be done at a cheaper price and there are, of course ample possibilities for every visitor to arrange his own sightseeing. However, when it comes to regular sightseeing tours comparable with the Congress tours in routes, transport facilities, meals and refreshments, guide services and so on, the prices are comparable to or higher than those offered by RESO for the Congress tours.

In response to our specific question regarding the price (75 Sw. kr) of the Congress excursion through the Stockholm archipelago, we have received the following information from RESO:

The participants will sail from Stockholm to Sandhamn and back to Stavsån by a rented ship. In order to save time, the trip from Stavsån back to Stockholm will be made by rented buses. The regular voyages from Stockholm to Sandhamn and back (entirely by ship, return price 24 Sw. kr) are subsidized by the City of Stockholm, not so the Congress tour. The latter, moreover, includes a first class lunch at Sandhamn, music and coffee on board, and the services of guides. RESO will be glad to answer further questions regarding this and other Congress tours.

It is our sincere hope that all participants of the Congress will find their stay in Stockholm pleasant and profitable, and we shall do our utmost to this end.

Yours faithfully,

LARS ERNSTER

9th International Congress of
Biochemistry,
c/o Svenska Kemistsamfundet,
Wenner-Gren Center, 6 tr.,
S-113 46 Stockholm

Pulsar Disclaimer

SIR,—The statement made by the authors at the end of "Periodicities in Seismic Response caused by Pulsar CP1133" by Dror Sadeh and Meir Meidav (*Nature*, **240**, 136; 1972), reading "We thank Professor C. L. Pekeris and the Applied Mathematics Department at the Weizmann Institute . . ." should not be construed as implying that I, or the Department of Applied Mathematics of the Weizmann Institute, are in any way responsible for the

views expressed by the authors in this paper.

Yours faithfully,

C. L. PEKERIS

Department of Applied Mathematics,
The Weizmann Institute of Science,
Rehovot

Cancer Chemotherapy

SIR,—I wish to present a strategy for cancer chemotherapy which, to my knowledge, has not been explicitly stated in print before.

Consider two classes of substances: class A substances kill only dividing cells, class B substances reversibly inhibit the division of normal cells but do not inhibit the division of malignant cells. The strategy is to first apply a class B substance; when it has taken effect, a class A substance is applied. Both are removed before unacceptable damage is done.

The key advantageous feature of this strategy lies in the protective effect of the class B substances. This will allow larger doses of more potent class A substances to be used more frequently and for longer periods.

Class A substances are well known among current chemotherapeutic agents. Do class B substances exist?

Yours faithfully,

DAVID ZIPSER

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Cold Spring Harbor, New York 11724

Announcements

Miscellaneous

THE Soviet State Prizes for science for 1972 were awarded as follows:

1. To Academician Boris Ivanovich Stepanov, Director of the Institute of Physics of the Byelorussian Academy of Sciences, Anatolii Nikolaevich Rubinov and Vasilii Andreevich Mostovnikov for work carried out in the Institute on the optical generation of complex organic compounds in solutions and the development of a new type of laser in connexion with this work.

2. To Dr Anatolii Filipovich Tulinov, Yurii Vladimirovich Melikov, Vatslavas Stanislavovich Kulikauskas, Grigori Arkad'evich Iferov and Grigori Pavlovich Pokhil of Moscow State University, Aii Aleksandrovich Puzanov of the Ural Polytechnic Institute, Bela Gabdulgaliyeva Akhmetova of the Kazakh State University, and Sarkis Arshavirovich Karamyan of the Joint Institute of Nuclear Research for their discovery and investigation of the shadow effect in nuclear reactions on monocrystals.

3. To Academician Evgenii Alekseevich Barbashin of the Byelorussian Academy of Sciences for his work on the stability of automatic control systems.

4. To Academician Ivan Pavlovich Alimarin, Academician Yurii Aleksandrovich Zolotov, Dr Mikhail Sergeevich Chupakhin, Yurii Vsevolodovich Yakovlev of the Institute of Geochemistry and Analytic Chemistry of the Soviet Academy of Sciences, Valentina Gavrilovna Goryushina and Dr Vsevolod Vasil'evich Nedler of the State Research Institute of the Rare Metals Industry and Dr Evgenii Aleksandrovich Bozhevol'nyi of the All-Union Research Institute of Chemical Reagents and Ultra-Pure Chemical Substances, for their work on the theory and new physicochemical methods of analysis of high-purity metals, semiconductors and chemical reagents.

5. To Dr Nikolai Mikhailovich Chirkov for his work on the kinetics of the polymerization and copolymerization of the α -olefines.

6. To Dr Petr Petrovich Timofeev of the Geological Institute of the Soviet Academy of Sciences for his work on the Jurassic deposits of Southern Siberia.

7. To Nikolai Aleksandrovich Krasil'nikov of the Institute of Microbiology of the Soviet Academy of Sciences for his work on the biology of the actinomycetes.

8. To Academician Nikolai Iosifovich Konrad and his team for their work in producing the "Large Japanese-Russian Dictionary".

9. To Dr Aleksei Andreevich Roda of the "V. V. Dokuchaev Soil Institute" for his work on soil humidity.

10. To Dr Vladimir Viktorovich Solodovnikov, Evgenii Pavlovich Popov, and Dr Viktor Vladimirovich Semenov of the Moscow "N. E. Bauman" Higher Tech-

nical College, Aleksandr Mikhailovich Letov, Dr Georgii Mikhailovich Ulyanov and Academician Boris Nikolaevich Petrov of the Institute of Control Problems, Germogen Sergeevich Pospelov of the Computer Centre of the Soviet Academy of Sciences, Dr Vil'ям Viktorovich Kazakevich of the Moscow Polygraphic Institute, Dr Lev Timofeevich Kuzin of the Moscow Institute of Engineering Physics, Dr Vyacheslav Vyacheslavovich Petrov of the Moscow Aviation Institute, Dr Yurii Ivanovich Topcheev and Dr Aleksandr Stepanovich Shatalov of the Dzhherzhinskii Institute of Military Engineering for their series of monographs on cybernetics.

11. To Dr Vladimir Tikhonovich Renne for his work on the theory, calculation and construction of electrical condensers.

Errata

IN the article "Bacterial Reduction of Arsenate in Sea Water", by David L. Johnson (*Nature*, **240**, 44; 1972), the following corrections should be made: paragraph 1, line 5, should read "... suggest that the concentration ratio is ..."; paragraph 2, line 6, superscripts 7-9 should be deleted; paragraph 3, line 3, 4.1% sucrose should read 0.1%; paragraph 3, line 9, the word sterile should be deleted.

IN the article "New Periodic Close Packings of Identical Spheres", by J. A. R. Coutanceau Clarke (*Nature*, **240**, 408; 1972), the following corrections should be made: line 4 of the last paragraph should read "... results from changing (11)s"; Table 1, column 4, items 1 and 2 should each read $\sqrt{3}m$; Table 2, column 7, item 15 should read $8(\sqrt{2} + \sqrt{3})$; Table 2, column 10A, item 10 should read $(11) \times \sqrt{3}/2$.

Reports and Publications

not included in the Monthly Books Supplement

Great Britain and Ireland

Scottish Marine Biological Association. Annual Report for the year ending 31st March, 1972. Pp. 71. (Oban: Scottish Marine Biological Association, Dunstaffnage Marine Research Laboratory, 1972.) [2610]

National Radiological Protection Board. Radiological Protection Bulletin, 1972, No. 1. Pp. 26. (Belmont: Sutton, Surrey: National Radiological Protection Board, 1972.) [2710]

Department of the Environment. Bibliography: Current Information on Maintenance. Part B: Design and Maintenance. Compiled by the Property Services Agency, Library Service. Pp. 36. (London: Property Services Agency Library, Lambeth Bridge House, 1972.) 50p. [2710]

Other Countries

Australia: Commonwealth Scientific and Industrial Research Organization. Division of Fisheries and Oceanography Technical Paper No. 33: Nutrient Enrichment of East Australian Coastal Waters. By D. J. Rockford. Pp. 17. (Melbourne: CSIRO, 1972.) [2310]

US Department of the Interior: Geological Survey. Professional Paper 495-E: Frontier, Cody, and Mesaverde Formations in the Wind River and Southern Bighorn Basins, Wyoming. By William R. Keefer. Pp. iii+23. Water-Supply Paper 1985: Ground-Water Resources of Orange and Ulster Counties, New York. By Michael H. Frimpter. Pp. v+80+4 plates. (Washington, DC: Government Printing Office, 1972.) [2410]

Smithsonian Contributions to Zoology. No. 97: The Systematics and Areal Distribution of Pelagic Cephalopods from the Seas off Southern California. By Richard Edward Young. Pp. iii+159 (38 plates). \$2. No. 124: A Review of the Family Synopiidae (=Tironidae), Mainly Distributed in the Deep Sea (Crustacea: Amphipoda). By J. Laurens Barnard. Pp. iv+94. \$1.25. (Washington, DC: Smithsonian Institution Press, 1972. For sale by US Government Printing Office.) [2410]

World Health Organization. Protection Against Ionizing Radiation: A Survey of Current World Legislation. Pp. 328. (Geneva: WHO; London: HMSO, 1972.) 28 Sw. francs; £2.80; \$7. [2410]

Nederlandse Vereniging voor Weer- en Sterrenkunde. Observations of Variable Stars, January-June 1972. (Report No. 22.) Pp. 5. (Groningen: Kapiteyn Astronomical Laboratory, 1972.) [2410]

The Museums of Man and Science/Die Museums van die Mens en Wetenskap. Pp. 16. (Johannesburg: Museums of Man and Science, 1972.) [2510]

Annals of the South African Museum. Vol. 59, Part 10: A Review of the Sepiidae (Cephalopoda) of Southern Africa. By Martina A. Roeleveld. Pp. 193-313+plates 35-45. R.10.80. Vol. 59, Part 11: Discard the Names Theriodontia and Anomodontia: a New Classification of the Therapsida. By L. D. Boonstra. Pp. 315-338. R.2.10. (Cape Town: South African Museum, 1972.) [2510]

New Zealand: Department of Health. Environmental Radioactivity Annual Report for 1971. Compiled by L. P. Gregory. (NRL-F/48.) Pp. 45. (Christchurch: National Radiation Laboratory, P.O. Box 25-099, 1972.) [2510]

Australian Academy of Science. Year Book, June 1972. Pp. 128. (Canberra, ACTL: Australian Academy of Science, P.O. Box 216, 1972.) [2610]

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